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AFRRI

Annual Research Report

COMPLETED
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ARMED FORCES RADIOBIOLOGY RESEARCH INSTITUTE
DEFENSE NUCLEAR AGENCY

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Research was conducted according to the principles enunciated in the "Guide for the Care and Use of Laboratory Animals," prepared by the Institute of Laboratory Animal Resources, National Research Council.

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INTRODUCTION

The Armed Forces Radiobiology Research Institute was established in 1961 as a subordinate command of the Defense Nuclear Agency. It is the primary Department of Defense facility for scientific research in the field of radiobiology and related matters. It conducts applied and basic research that is essential for the operational and medical support of the Department of Defense. The work is carried out by five scientific departments as listed below:

Behavioral Sciences: Effects of ionizing radiation, chemicals, and drugs on performance.

Biochemistry: Elucidation of mechanisms of injury, repair, and protection from the effects of ionizing radiation alone or in combination with other agents; development of improved methods to detect and quantify the severity of radation injury.

Experimental Hematology: Investigation of radiation injury of bone marrow; development of therapy for damage from intermediate radiation doses; determination and treatment of injuries caused by combined effects of radiation, blast, and burns.

Physiology: Research on cellular, tissue, and whole-animal models to determine physiological and biophysical changes resulting from radiation either alone or in combination with drugs or other chemicals.

Radiation Sciences: Operation, maintenance, and quality control of all AFRRI radiation source radiation dosimetry and estimation of tissue doses at various depths in different kinds of tissues; development and use of nuclear medicine and magnetic spectroscopic techniques for determining radiation damage in animals and model systems.

The results of this broad multidisciplinary program are summarized in this report. In addition, much of the work is published in the scientific literature, where it contributes significantly to the body of radiobiological knowledge, as well as in AFRRI scientific and technical reports.

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BEHAVIORAL SCIENCES DEPARTMENT

The Behavioral Sciences Department conducts investigations assessing the effects of ionizing radiation, chemicals, and drugs on combat performance. This program encompasses a spectrum of multidisciplinary approaches in experimental animal models, ranging from operant conditioning techniques to methods used in physiological psychology, neurochemistry, and neurophysiology. The Department addresses the specific behavioral consequences of exposure to ionizing radiation, and explores the biological mechanisms responsible for radiation-induced behavioral decrements. Collaborative efforts are pursued with other AFRRI departments, the Naval Research and Development Command, universities, and other Federal Government laboratories.

The Department has two Divisions: the Experimental Psychology Division and the Physiological Psychology Division. The Experimental Psychology Division uses behavioral and electrophysiological models to determine the conditions under which ionizing radiation and militarily relevant chemicals can degrade combat performance. Behavioral tasks that model specific aspects of cognitive and physical combat performance are used to determine the radiation levels that affect combat effectiveness. The information from these studies is compiled into a database, which provides rapid retrieval, overall summary analyses, and development of extrapolated models applicable to battlefield conditions. Research in behavioral toxicology quantitates the changes in behavioral and neurophysiological capabilities due to exposure to chemicals and radiations that may be present in the military environment. This work uses a battery of tests designed to provide information on toxic dose levels and/or mechanisms by which these environmental hazards affect behavior.

The Physiological Psychology Division explores the mechanisms by which ionizing radiation and chemical toxins disrupt behavior. Behavioral, physiological, and neurochemical approaches are predominant. This information can be used to develop methods of preventing performance decrement.

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IONIZING RADIATION DECREASES VERATRIDINE-STIMULATED SODIUM UPTAKE IN RAT BRAIN SYNAPTOSOMES

Principal Investigators: H. N. Wixon and W. A. Hunt

The central nervous system has generally been considered to be relatively resistant to the direct effects of ionizing radiation. Electrophysiological studies have suggested that ionizing radiation can induce changes in the bioelectric properties of nerve cells only at supralethal doses. Some investigators have shown an enhancement of neuroexcitability after 10-krad doses of radiation, while others have reported no effect on action potentials, conduction velocity, or membrane resistance. At very high supralethal doses above 10 krad, a decrease in excitability was reported by all investigators.

Excitable cell membranes are characterized, in part, by their ability to generate action potentials via time- and voltage-dependent changes in membrane conductance to sodium and potassium ions. The permeability pathways for the two ions are distinct; i.e., there exist, within the membrane, functionally defined ion channels that are specific and exclusive for sodium and for potassium. The purpose of the present study was to clarify the effects of ionizing radiation on the properties of a discrete entity of neural function, the sodium channel. Veratridine, a neurotoxin, selectively opens sodium channels. By measuring the rate of sodium uptake in the presence of this toxin, the integrity of the channel can be inferred.

Synaptosomes were irradiated with 10-100,000 rad of high-energy electrons. Using a veratridine concentration of 10 μM, uptake of sodium-22 was significantly reduced in a dose-dependent manner. The decrease in uptake produced by irradiation ranged from 13% to 60%. Using different concentrations of veratridine, it was found that the response to maximal veratridine stimulation was reduced in a dose-dependent manner by radiation, but with no apparent shift in the concentration-response curve. Nonspecific sodium uptake in the presence of 1 μM tetrodotoxin was unaffected by radiation at the doses used. These data suggest that radiation disrupts the normal functioning of the sodium channel directly, rather than through an alteration in the affinity of sodium for the channel.

Radiation may in some way reduce the number of sodium channels that can open upon stimulation, or radiation may induce a conformational change that restricts the extent to which the sodium channels can open.

A radiation effect such as observed in this series of experiments suggests a sensitivity not heretofore demonstrated in the central nervous system of mammals to acute sublethal radiation doses. If this is the case, it indicates the possibility of radiation-induced disruption of a very fundamental central nervous system process that could affect the behavior of individuals at far lower doses than previously believed, perhaps even at dose levels now commonly used for therapeutic purposes. Further experiments involving discrete receptor sites in the sodium channel may provide additional information on the mechanism by which radiation exerts its effect, and on the biological significance of these findings.

* * *

ROLE OF THE AREA POSTREMA AND SOME HUMORAL FACTORS IN LEARNING RADIATION-INDUCED TASTE AVERSION

Principal Investigators: B. M. Rabin and W. A. Hunt
Technical Assistance: J. F. Lee

This work has been concerned with attempting to determine the mechanisms by which exposure to ionizing radiation can produce changes in the behavior of an organism. The specific behavior being used is the conditioned taste aversion (CTA). CTA is produced by pairing a normally preferred solution with a toxic stimulus, so that the animals will avoid that solution at a subsequent presentation. The CTA paradigm is being used as a behavioral marker to provide a means for understanding how exposure to sublethal levels of radiation (approximately 100 rad) can produce changes in brain activity and behavior. Also, since the CTA is one mechanism by which an animal protects itself from the ingestion of poisons, it may serve as a model for emesis in rodents (which do not vomit), and allow for rapid and inexpensive accumulation of data.

The first experiment involved the attempt to analyze the role of the area postrema in the acquisition of CTA, since the area postrema is the chemoreceptive trigger zone for emesis. Destruction of this brain stem region disrupts the emetic responses to a variety of treatments, including radiation. Lesions in this region in rats resulted in an attenuation of radiation-induced CTA, indicating that CTA and emesis may use the same brain area for their expression. Also, because the area postrema monitors the blood for toxins, our data are consistent with the hypothesis that radiation induces the release of a humoral factor to which it is sensitive and which, in turn, functions to mediate some of the behavioral responses to exposure to ionizing radiation.

Given the common involvement of the area postrema in mediating both the emetic and CTA responses to toxic stimulation, the next experiment examined the possibility that antiemetic drugs might modulate the CTA response to ionizing radiation. Rats were administered the antiemetics cyclizine, prochlorperazine, or trimethobenzamide before radiation exposure. Pretreatment with drugs, in the doses used, had no attenuating effect on the acquisition or recall of a CTA induced by radiation.

Finally, since an intermediate dose of radiation (650 rad) can affect the secretion of pituitary/adrenal hormones, an experiment was designed to review the possibility that these hormones might mediate the acquisition of a radiation-induced CTA. The subjects for the experiment were hypophysectomized rats and their littermate controls. Removal of the pituitary gland did not attenuate the CTA produced by radiation, suggesting a lack of significant involvement of their hormones in CTA acquisition.

* * *

APPLICATION OF A NEW PROCEDURE FOR ANALYSIS OF BIOGENIC AMINES AND THEIR METABOLITES BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY: A PRELIMINARY RADIATION STUDY

Principal Investigators: W. A. Hunt and T. K. Dalton
Technical Assistance: K. Alberstadt

This laboratory has recently developed a new, fully automated method for the determination of several biogenic amines and their metabolites by high performance liquid chromatography (HPLC) using electrochemical detection. With a sensitivity of as low as 500 fmoles, this method can quantitate up to 15 compounds from a single sample. The applicability of this method for analyzing these substances in brain tissue and biological fluids was undertaken.

Various areas of the rat brain were analyzed using the HPLC method. They included gross brain areas as well as discrete brain nuclei as follows: cerebral cortex, cerebellum, caudate nucleus, hypothalamus, hippocampus, globus pallidus, substantia nigra, nucleus accumbens, lateral hypothalamic nucleus, and raphe nucleus. Values obtained for the different constituents were consistent from sample to sample, and could be reliably obtained from a single tissue sample. In addition to brain samples, cerebrospinal fluid from cats and rabbits and urine from rats could be analyzed successfully.

Previous data from our laboratory suggest that dopaminergic mechanisms are altered after a single dose of ionizing radiation (1). A 10-krad dose of high-energy electrons elevates dopamine release in the caudate nucleus, an effect that follows the time course of early transient incapacitation. Using the above analytical technology, experiments were begun to further explore the role of dopamine in the effects of radiation on the brain, and to expand the investigations to areas of the brain other than the caudate nucleus and to other neurotransmitters.

Preliminary studies were performed to determine the effect of 20 krad of gamma radiation on various biogenic amines and their metabolites in several areas of the rat brain. Results from a limited number of animals suggests that norepinephrine concentrations are reduced in the hypothalamus and cerebellum.

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SENSORY AND MOTOR CONTRIBUTIONS TO POST-EXPOSURE BEHAVIORAL DECREMENTS

Principal Investigator: W. F. Burghardt
Technical Assistance: B. A. Dennison

Previous research on the effects of ionizing radiation on behavior has provided information that makes possible some lethality estimates, characterization of behavioral incapacitation on several different tasks, and information concerning the interaction of fatigue and exposure to radiation. To provide a better characterization of behavioral effects, more information is required on the relative contributions of the motor and sensory systems to this behavioral effect, and on the importance of the type of task required of the organism. This could be achieved by the simultaneous measurement of performance and signal utilization on several concurrent tasks of differing importance.

During FY 82, setup and psychopharmacological validation of such a behavioral task was substantially completed using a rodent model. This paradigm consisted of a bar-press electrical footshock avoidance task combined with a bar-press task on another lever, which afforded the subject the opportunity to provide itself with both a light (as a response-feedback cue) and a tone (which served as a warning signal for shock). Results of studies with drugs that stimulate or depress the central nervous system provided results consistent with their known mechanisms of action.

Preliminary data on this task after exposure to 10 krads of cobalt-60 radiation at approximately 6.6 krads per minute demonstrated a 20- to 30-minute period of diminished task performance, as measured by increased density of shocks received, increased latency to respond to escape shocks, and increased latency to respond to a warning tone preceding shock presentation, along with profound differential changes in response frequency on the two tasks used. During recovery from this performance decrement, although responding on the avoidance lever increased (but never reached preirradiation levels), the task efficiency was still impaired, and responding for the warning signals was reduced to extremely low levels. These results correlate with previous characterizations of early transient incapacitation (ETI), and extend this information to suggest that the behavioral effect obtained is not primarily of motor origin, but involves the way in which an organism responds to external cues. It is also dependent on the nature of the task on which performance is measured.

Two additional pilot studies were initiated. The first one addressed the role of endogenous opiates in the production of behavioral decrement after radiation through the possible induction of analgesia. The other was the possibility of interactive changes in brain catecholamines in response to exposure to ionizing radiation and environmental stressors as related to the postulated role of these neurotransmitters in the production of motor dysfunction in the brain. Results at this point are inconclusive, but support the position that postirradiation behavioral decrements are neither primarily motor nor opiate mediated.

* * *

CONVERSION OF RADIATION DOSES IN THE AFRRI BEHAVIORAL DATA BASE TO BATTLEFIELD-EQUIVALENT DOSES

Principal Investigators: R. W. Young, C. G. Franz, S. G. Levin, and W. E. Jackson
Technical Assistance: W. S. Bice and K. P. Ferlic

Casualty criteria for military personnel exposed to ionizing radiation are primarily based on performance studies in monkeys. If an equivalent midline tissue dose (MTD) is delivered to the head of both a monkey and man, it is assumed that each will have a similar decrement in performance. However, because the anatomies of their heads differ, it is necessary to expose man to a different free-in-air (FIA) dose to have an MTD equivalent to the monkey's. Doses for monkeys exposed to ionizing radiation in the AFRRI TRIGA reactor are reported as the MTD at either the head or thorax. However, the U.S. Army bases its casualty criteria on the FIA dose at the midhead of personnel exposed to the spectrum of a nuclear weapon. The purpose of this study is to convert the MTD's of over 600 monkeys on the AFRRI behavioral data base to FIA doses and radiation spectra more meaningful to operational planners.

The first step in the conversion is to standardize the dosimetry for the studies in the data base. Because the MTD varies with the size of the subject, phantoms 4, 5, and 6 inches in diameter were developed by the AFRRI Dosimetry Division to model small, medium, and large monkeys. Work is in progress to repeat the dosimetry for each of the three sizes, at each of the different room positions and radiation fields in which monkeys were exposed. MTD's are measured in the phantoms at the midhead and midthorax, and the FIA doses are measured at the middle of the back and head. From these doses are calculated the tissue-to-air ratios (TAR) and the FIA-thorax-to-FIA-head ratios. For a given radiation quality and exposure position in the TRIGA reactor, the FIA ratio is constant for any dose.

After the dosimetry calculations have been completed, the MTD at the midhead for monkeys exposed at the midthorax can be estimated by

$$\frac{MTD_{tm}}{TAR_{tp}} = FIA_{tm}$$

$$FIA_{tm} \times \frac{FIA_{tp}}{FIA_{hp}} = FIA_{hm}$$

$$FIA_{hm} \times TAR_{hp} = MTD_{hm}$$

where hm = head of monkey, hp = head of phantom, tm = thorax of monkey, and tp = thorax of phantom.

Because the field is not uniform over the distance between the head and thorax, it is necessary to adjust the FIA dose received at the thorax with the FIA-thorax-to-FIA-head ratio (determined from the phantom studies) to calculate the FIA dose at the head.

After the midthorax doses in the monkey studies have been changed to midhead doses, each must be converted to the FIA midhead equivalent for man. Previous work at the U.S. Army Nuclear and Chemical Agency (1) used the T-65 estimates of the Hiroshima and Nagasaki weapons (2) to compute a conversion factor. As part of this study, personnel at the Oak Ridge National Laboratories have calculated conversion factors for the FIA battlefield doses using actual current weapon spectra and a sophisticated man-head model. These conversion factors will be used in future model development and reports to DoD agencies.

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2. Milton, P. C. and Shohoji, T. Tentative 1965 dose estimation for atomic bomb survivors. Atomic Bomb Casualty Commission, Technical Report TR-168, January 1968.

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ANALYSIS AND MODEL DEVELOPMENT OF PERFORMANCE DATA ON THE AFRRI BEHAVIORAL DATA BASE

Principal Investigators:	R. W. Young, C. G. Franz, S. G. Levin, and W. E. Jackson
Technical Assistance:	W. S. Bice and K. P. Ferlic

Monkeys trained to a visual discrimination task have been exposed to whole-body ionizing radiation at doses ranging from about 650 to 15,000 rads (referenced to midline thorax). Performance data from these radiation studies have been entered into a computerized data base for rapid retrieval, analysis, and model development. An important outgrowth of the data base is an estimation of the degree of performance decrement or incapacitation that occurs at doses not tested. By fitting the data to probit functions, an estimate can be made of the percentage of animals incapacitated at any time postirradiation.

Initially, both untransformed and log transformation of doses were used in the probit functions to fit the performance data. The results were unsatisfactory because of the nonlinearity of the incapacitation phenomenon. Other transformations were used to linearize the dose-response function. The most satisfactory was the Box-Cox transformation, as follows:

$$\text{Transformed dose} = \frac{(\text{Dose} - K) \quad - 1^{\lambda}}{-\lambda}$$

With the Box-Cox transformation, it is possible to analyze the performance data in minute intervals and to improve the resolution of the model. Previous models have assumed that if a group was incapacitated at any time during the first 2 hours following irradiation, it was incapacitated during the entire 2 hours.

Performance data from animals irradiated in the AFRRI reactor in fields with n/g ratios of 2:5 (normal TRIGA field) and 3:1 (TRIGA field moderated by 2 inches of lead) have been analyzed using the Box-Cox transformation. Because most interest has been centered on early transient incapacitation (ETI), only responses for the first 2 hours following radiation were studied. Future efforts include determining optimal data-smoothing methods, analyzing data from radiations in n/g fields of 10:1 and 1:10, and extending the analysis period from 2 hours postirradiation to 200 hours. By increasing the period of analysis, the late transient incapacitation and the permanent complete incapacitation can be studied in addition to ETI.

* * *

EFFECT OF COBALT-60 RADIATION ON THE VISUAL EVOKED POTENTIALS OF RATS

Principal Investigators: R. M. Cartledge and R. W. Young

Performance of monkeys trained to a visual discrimination task is often degraded for several hours following exposure to lethal doses of ionizing radiation. The performance decrement is more severe if the radiation field includes a high proportion of gamma photons (1). One of the postulated mechanisms for this phenomenon, called early transient incapacitation (ETI), is the precipitous decline in cerebral blood flow that immediately follows radiation (2,3). Because visual function is impaired following changes in blood oxygen levels (4) and is critical to the successful performance of many military tasks, the immediate effects of gamma radiation on visual function were studied using electrophysiological methods. This report reviews the preliminary findings of this study.

Visual evoked potentials (VEP's) have been collected from three adult, male Sprague-Dawley rats every 5 minutes for 2 hours following whole-body exposure to cobalt-60 radiation. Recording electrodes were surgically implanted over the occipital cortex at least 2 weeks before the irradiation. VEP's were collected from awake subjects confined in a Plexiglas holder positioned 20 cm from a Grass PS22 photostimulator. Flashes were presented once every second at an intensity of 16 X on a 1-16 X scale. There were 250 flashes per average. Analysis included measuring the latencies and amplitudes (referenced from a prestimulus baseline) of individual peaks, and measuring the peak-to-peak amplitudes between adjacent components. A sham irradiation conducted 24 hours before the exposure served as a baseline.

The results to date indicate a dramatic decrease from baseline in the amplitudes of the early peaks of the VEP in each of the subjects immediately following exposure to 13,000 rads delivered at 2,000 rads per minute. The greatest decrease occurred during the first 20 minutes after irradiation, and then the amplitudes gradually returned to baseline values at the end of 2 hours. These data suggest that a decrease in visual function occurs immediately after exposure to high doses of ionizing radiation, that its time course is similar to that observed for ETI, and that decrements in the visual discrimination task following radiation may be related to visual dysfunction.

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4. Kayama, Y. Evoked potentials of the central visual system during and after hypoxia in cats. Electroencephalography and Clinical Neurophysiology 36: 619-628, 1974.

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COMPARISON OF ACCELEROD AND ROTAROD SENSITIVITY IN DETECTING ETHANOL- AND ACRYLAMIDE-INDUCED PERFORMANCE DECREMENT IN RATS

Principal Investigators: V. Bogo and R. W. Young, *AFRRI*
T. A. Hill, *Naval Medical Research Institute*
Technical Assistance: C. A. Boward and G. G. Kessell, *AFRRI*

The relative sensitivity of two rotating rod techniques in detecting performance decrement in rats was assessed after treatment with either ethanol or acrylamide (1). Performance on the rod during acceleration at approximately 1 rpm/sec (accelerod) was compared to that for the same rod operated at a constant speed of 20 rpm (rotarod). Rats trained to either task received a single oral dose of ethanol (0.5, 1.0, 1.5, or 2.0 g/kg) or a series of intraperitoneal doses of acrylamide (25 or 50 mg/kg/day) before testing.

Accelerod performance was significantly more disrupted at lower doses for longer periods of time after ethanol ingestion than was rotarod performance (Table 1). Similarly, task disruption following repeated injections of acrylamide appeared sooner when subjects were tested on the accelerod (Table 2). A higher proportion of the naive subjects were successfully trained, and the mean time for training to minimum performance standards was significantly less using the accelerod. The greater sensitivity of the accelerod in detecting neurotoxicity is attributed primarily to the fact that this test provides a continuous measure of the upper limit of performance rather than the quantal measure that defines rotarod performance. The validation of the accelerod as a sensitive measure of motor performance is complete. This technique will next be used to assess the effects of radiation on motor performance in rats.

REFERENCE

1. V. Bogo., T. A. Hill, and R. W. Young. Comparison of accelerod and rotarod sensitivity in detecting ethanol- and acrylamide-induced performance decrement in rats: Review of experimental considerations of rotating rod systems. Neurotoxicology 2: 765-787, 1981.

Table 1. Comparison of Significant Ethanol-Produced Decrement in Accelerod and Rotarod Performance for 5 Hours of Testing

Dose level of ethanol (gm/kg)	Accelerod	Rotarod
0.5	No significant effects	No significant effects
1.0	Significant effect across the 5 hours of testing	No significant effects
1.5	Significant effect across the 5 hours of testing	No significant effects
2.0	Significant effect for the 30- to 270-min test trials and across the 5 hours of testing	Significant effect for the 60- to 150-min test trials and across the 5 hours of testing

Table 2. Significant Acrylamide-Produced Decrement in Accelerod and Rotarod Performance in Terms of Cumulative Dose and Day to Effect

Dose Group (mg/kg/day)	Accelerod	Rotarod
	Cumulative Dose to Significant Effect	
25	200	250
50	100	200
	Cumulative Day to Significant Effect	
25	8	10
50	2	4

* * *

AUTOMATED GENERATION AND ANALYSIS SYSTEM FOR INHALATION TOXICOLOGY STUDIES

Principal Investigators: V. Bogo, R. W. Young, and R. M. Cartledge, *AFRRI*
T. A. Hill, *Naval Medical Research Institute*

DoD has been assessing for several years the feasibility of producing synthetic fuels extracted from coal and shale deposits. Questions as to their feasibility include not only their operational suitability but also their potential health hazards to military personnel. Studies have been conducted in rats to characterize the toxicity of several synthetic fuels following subchronic inhalation exposure. The purpose of this report is to describe the systems used to generate the fuel vapors and automatically assay the exposure concentrations.

The exposure system consists of four 30-liter Leach chambers, an all-glass-and-Teflon vapor generator with concentration controller, and an exposure environment sampler and analyzer (Figure 1). The inhalation chambers (two control and two experimental) are glass bell jars with removable stainless-steel grid floors and 8-inch marine bronze port lights for airtight front closures. The chambers are ventilated at 10 liters per minute with compressed air that has been dehumidified and then filtered through activated charcoal and a Type 4X molecular sieve.

The vapor generator uses a countercurrent flow of liquid fuel versus air within a heated glass cylinder. The fuel is pumped into the top of the cylinder and spreads in a thin film over the entire inner surface as it flows down over a series of helical ridges. Fuel vaporized from the heated surface mixes with clean air coming into the bottom of the cylinder, and exits via a tee fitting at the top. The cylinder is heated by a Nichrome wire coil (50.87 feet of 25-gauge, uncoated wire in a 3/8-inch coil, total resistance of 177 ohms) wound in the helical indentation of the outer surface. The temperature of the generator is maintained at 50°C by a proportional controller that receives temperature feedback from a thermistor detector inserted into the exit tee. Approximately 25% of the airflow through the system is saturated with fuel vapor. The vapor-air mixture is diluted to the appropriate concentration at the exit tee, and is then piped to the exposure chambers.

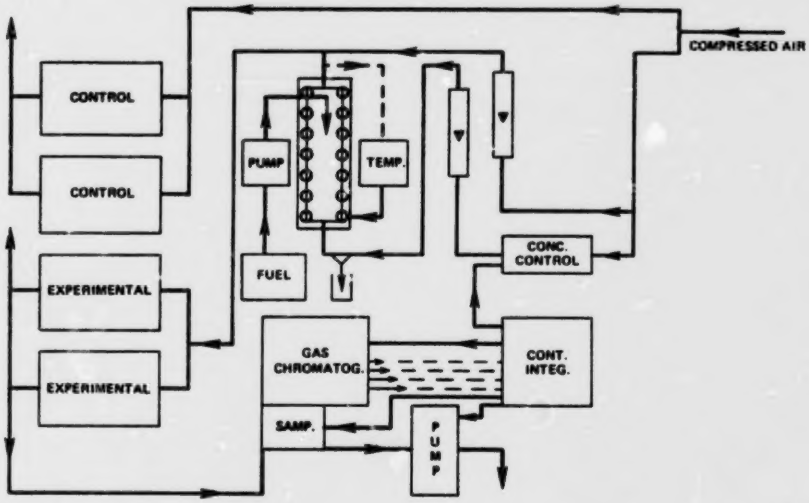

Figure 1. Schematic of inhalation exposure system. Dehumidified and purified air flows either directly to control chambers or via concentration control dilution valve (conc control) and bypass lines to top and bottom of vapor generator. Liquid from fuel reservoir (fuel) is supplied by fuel pump (pump) to generator. Excess fuel is trapped and drained off at bottom of generator. Generator temperature is maintained by proportional controller (temp). Controlling integrator (cont integ) coordinates operation of gas chromatograph (gas chromatog), chamber environment-sampling valve (samp), chamber environment-sampling pump (pump), and concentration control dilution valve. Detector output from gas chromatograph is analyzed by the controlling integrator.

The amount of total hydrocarbons, oxygen, and carbon dioxide within the chambers is monitored at 12-minute intervals by a microprocessor-controlled gas chromatograph (GC). A user-programmable (BASIC) computing integrator coordinates the operation of a sampling pump, the selection of GC detectors, the cycling of the column-switching valve, and the start signal to the GC during a sampling and assay sequence. The combined sampling valve and column-switching system (Figure 2) has been configured to alternately detect a consolidated hydrocarbon peak by flame ionization and to assay fixed gases by thermal conductivity. In the

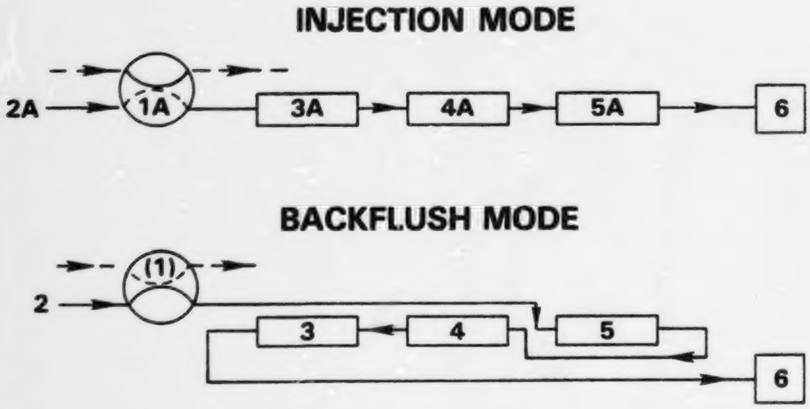

Figure 2. Schematic of chromatography column and sampling valve configuration. In injection mode (upper panel), chamber air contained in sample loop (1A) is flushed with helium carrier gas through columns 3A, 4A, and 5A in sequence. Components of sample mixture partition between the three columns, with hydrocarbons remaining in 3A, carbon dioxide in 4A, and combined oxygen-nitrogen peak in 5A. Switching to backflush mode (lower panel) reverses the flow in 3 and 4 but allows components in 5 to continue through full length of column. Hydrocarbons are flushed off column 3 into detector, followed by carbon dioxide from column 4 and finally by totally separated oxygen and nitrogen peaks.

injection mode (Figure 2, upper panel), chamber air contained in sample loop 1A is flushed by the helium carrier gas 2A into a series of three columns. Column 3A retains hydrocarbons in 1% Dexsil 300 on 100/200-mesh Gaschrom Q in 18 inches of 1/8-inch-diameter stainless steel. Carbon dioxide is retarded in column 4A on 80/100-mesh Poropak Q in a 10-foot x 1/8-inch-diameter stainless-steel column. The combined oxygen and nitrogen peak passes into a 5-foot x 1/8-inch stainless-steel column (5A), packed with 30/60-mesh Type 5A molecular sieve. Before carbon dioxide begins to emerge from column 4A, the system is switched to the backflush mode (Figure 2, lower panel). The flow of carrier gas continues forward through column 5, but reverses in both columns 3 and 4. The hydrocarbons elute first, followed by carbon dioxide, oxygen, and nitrogen. The column effluent is split between the flame ionization detector and the hot-wire detector in a flow ratio of 2.50:1. The single-channel integrator alternates between the two detectors, and automatically adjusts the timing of the backflush valve to maintain appropriate peak shape and elution time. The total carrier flow rate is 35 ml/min, the valve and column ovens are maintained at 80°C, and the hydrocarbons and fixed gases analyses are reported every 12 minutes. A typical chromatogram and assay report is shown in Figure 3.

```
X00182INSJP5-RUN             04 15:11:49

FILE 1     METHOD  5.     RUN  27     INDEX  27

ANALYST: HILL

SAMPLE   1     SHALE JP-5

NAME              CONC       RT      AREA BC      RF       RRT

SHALE JP-5      969.415     1.36      4279 01      4.414   0.21
CARBN DIOX        0.413     6.04       427 01   1034.9     0.934
OXYGEN           21.213     6.47     18370 01    866.      1.
NITROGEN         78.377     7.07     70806 01    903.4     1.

TOTALS         1069.418              93882

STATISTICS REPORT     INDEX  27

FILE ENTRY     AVERAGE      REL SD %      STD DEV      VARIANCE

SHALE JP-5     909.925        6.721       61.157      0.3740↑+04
CARBN DIOX       0.336        9.262        0.031      0.9699↑-03
OXYGEN          21.3          0.396        0.084      0.7120↑-02
NITROGEN        78.383        0.288        0.226      0.5104↑-01


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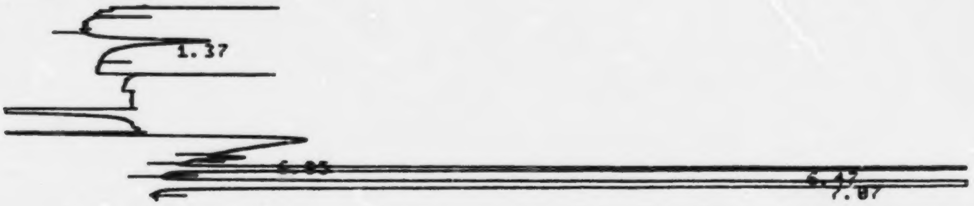

Figure 3. Typical chromatogram and component assay with statistical update for first 27 samples. Concentration (conc) for named constituents is given in mg/cubic meter for shale JP5 and in percent for others. Remaining columns contain values for retention time of peak (RT), integrated area under peak (area), baseline correction code (BC), detector response factor (RF), and relative retention time for detected peaks (RRT).

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BIOCHEMISTRY DEPARTMENT

The main objectives of the research conducted in the Biochemistry Department are (a) to elucidate the biochemical mechanisms of injury, repair, and protection of the mammalian organism from the effects of radiation, either alone or in combination with other toxic agents, and (b) to develop more effective, sensitive, and easy-to-perform methodologies to detect and quantify the severity of radiation-induced injury to man.

The Department is organized into three Divisions: Physiological Chemistry, Molecular Biology, and Immunological Chemistry.

The Physiological Chemistry Division is primarily concerned with identifying and developing biochemical indicators of radiation injury. Major emphasis is placed on changes in serum glycoproteins, trace metals, and indicators of radiation-induced membrane lipid peroxidation. The effects of radiation on the immune response of the mammalian organism has also been under investigation. We have extensively studied the role of various immunostimulants alone or in combination with radioprotectant drugs in alleviating or preventing radiation-induced injury. Various dietary adjuvants such as vitamins E, C, and A are being tested for their radioprotective properties. The effect of ionizing radiation on bone and the formation of bone marrow has been studied.

The research objective of the Molecular Biology Division is to elucidate the biochemical mechanisms of injury induced by toxic agents such as ionizing radiation alone or in combination with toxic chemicals, with emphasis on commercially available anticholinesterases. Methods for more effectively protecting the mammalian organism from the effects of these agents are under extensive investigation. We are also identifying the sequence of biochemical events that lead to radiation-induced damage of cellular membranes. We are investigating the role of various radioprotectants in preventing or alleviating this damage. The effects of radiation on histamine and prostaglandin release and the mechanisms responsible for this release are being systematically investigated.

The Immunological Chemistry Division is concerned with isolating hematopoietic and progenitor cells and measuring their potential as modifiers of radiation-induced injury. We have investigated the nature of the interaction of stromal tissue and hematopoietic cells, as well as the importance of postirradiation hematopoietic regeneration.

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EFFECTS OF ^{60}Co GAMMA RADIATION ON SYNTHESIS OF PROSTAGLANDINS $F_{2\alpha}$, E, AND THROMBOXANE B_2 IN LUNG AIRWAYS OF GUINEA PIG

Principal Investigators: L. K. Steel and G. N. Catravas
Technical Assistance: I. K. Sweedler

The prostaglandins (PG) have been implicated in the modulation of pulmonary vascular and airway function. We have directed efforts toward elucidating the contribution of the lung bronchial tree in the bronchopulmonary functional response to radiation insult. The present investigation was designed to determine the effects of ^{60}Co gamma radiation on levels of lung bronchial airway prostaglandins and thromboxane. Tissue capacity to synthesize PG in response to the exogenous addition of histamine or the calcium ionophore A23187 was also studied.

At 1 hour to 14 days after total-body exposure of guinea pigs to 300 rads ^{60}Co gamma radiation, changes were detected in PG concentrations in bronchial airway tissues (Figure 1). At 3 hours postexposure, tissue levels of prostaglandin E were significantly elevated, while at 48 hours, transiently elevated levels of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) were observed. By 72 hours, levels returned to control values. Airway synthesis of thromboxane B_2 in irradiated animals did not differ from that in controls. Assessment of the capacities of bronchial airway preparations to respond to (a) H-1 receptor stimulation by the exogenous addition of histamine or (b) stimulation of transmembrane divalent-cation transport by ionophore, revealed alterations in the amount and type of PG generated, which varied with time postirradiation.

These findings suggest that a number of enzymatic processes may be affected and that the levels of prostaglandin or thromboxane B_2 might be related to the radiosensitivity of certain organs or cell types. In view of the possible contributions of prostaglandins to some of the symptoms following irradiation, efforts are being made to delineate the mechanisms that regulate the types and amounts synthesized, and to clarify their role in the pathologic events of radiation injury.

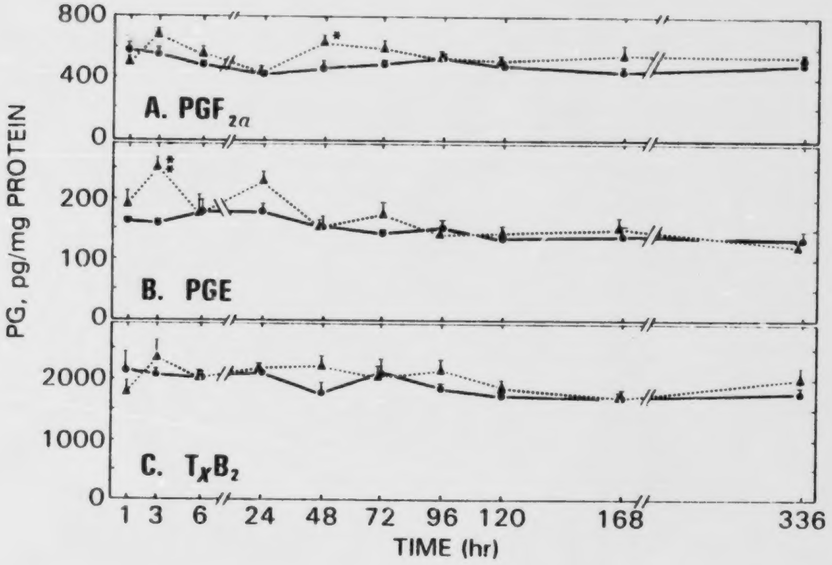

Figure 1. Effect of 300 rads of cobalt-60 gamma radiation on levels of basal PGF$_{2\alpha}$, PGE, and TxB$_2$ in tissues of guinea pig airway lung. Airway replicates were incubated in Tyrode's buffer only. Prostaglandin (PG) levels are given as pg/mg guinea pig airway protein. (A) PGF$_{2\alpha}$ levels. (B) PGE levels. (C) TxB$_2$ levels. Sham-irradiated (control) release, ●——●; irradiated, ▲·······▲. Means ± SEM are given; SE indicated by vertical lines. *$p < 0.05$; **$p < 0.001$ (toward corresponding control). n = 18.

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MODULATION OF HISTAMINE RELEASE IN THE RAT MAST CELL BY TRIFLUOPERAZINE

Principal Investigators:	D. E. McClain, M. A. Donlon, and G. N. Catravas
Technical Assistance:	W. W. Wolfe and K. Lambright

The mechanism by which radiation induces histamine release and early transient incapacitation (ETI) is not known. Mast cells contain the body's largest stores of peripheral histamine. As a result of radiation, this dispersed cellular system releases not only histamine but also more than 15 other chemical mediators and bioactive compounds, which exert profound pathophysiological effects.

As part of our continuing study to understand the mechanism of radiation-induced histamine release, especially with regard to calcium metabolism, we exposed mast cells to the calmodulin-binding drug trifluoperazine, and measured its effect on the histamine release stimulated by various releasing agents (1). Histamine release is a calcium-dependent process, and calmodulin (a calcium-binding protein) is involved in a wide variety of calcium-dependent processes in cells.

In this work we show that trifluoperazine modifies the histamine release stimulated by Compound 48/80 and A23187 in the mast cell. Mast cells were incubated at 37°C for 2 minutes with 25, 50, and 100 nM trifluoperazine. The drug was then removed by washing. The cells were then exposed to 1.0 μg/ml Compound 48/80 or A23187 for 5 and 10 minutes, respectively. Figure 1 shows the results of these experiments. Preincubation of the cells with trifluoperazine led to an inhibition of histamine release, stimulated by both releasing agents.

Trifluoperazine appears to be somewhat toxic to the mast cell at higher concentrations than those used in these experiments. Concentrations of trifluoperazine from 25 to 100 nM did not affect cell viability, cell number, or unstimulated histamine release. Therefore, the inhibition of stimulated histamine release was not a cytotoxic effect of the drug.

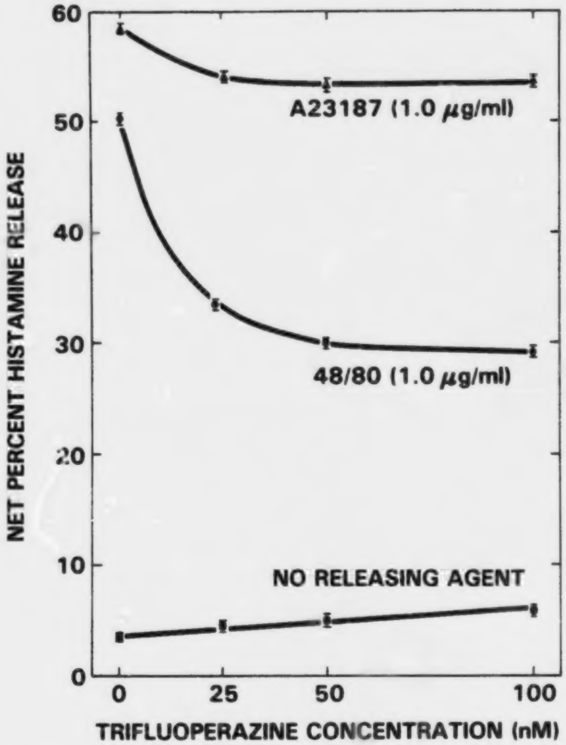

Figure 1. Effect of trifluoperazine (TFP) on 48/80 and A23187 directed histamine release in rat peritoneal mast cells. Cells were exposed to various concentrations of TFP for 2.0 minutes at 37°C and then washed twice with TFP-free buffer. The TFP-preincubated cells were exposed to 1.0 µg/ml 48/80 or A23187 for 5 and 10 minutes, respectively.

The net decrease in the histamine release observed in the presence of trifluoperazine may reflect the inhibition of a calmodulin-sensitive event in the release process.

REFERENCE

1. McClain, D. E., Donlon, M. A., and Catravas, G. N. Trifluoperazine modulation of histamine release in the rat mast cell. Federation Proceedings 41: 505, 1982.

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EFFECTS OF MIXED NEUTRON-GAMMA RADIATIONS ON GENERATION OF PROSTAGLANDIN AND THROMBOXANE IN LUNG TISSUES OF GUINEA PIG

Principal Investigators: L. K. Steel and G. N. Catravas
Technical Assistance: I. K. Sweedler

Results of previous experiments concerned with the in vivo effects of ionizing radiation on parenchymal and airway lung tissues indicated that when guinea pigs were exposed to 50, 150, or 300 rads of ^{60}Co radiation, marked alterations in the concentrations of basal prostaglandin and thromboxane occurred. The objective of this study is to determine the effects of a different quality of ionizing radiation, specifically radiation rich in neutrons, on the cyclooxygenase system in lung tissues. We also investigated the capacity of tissues to generate prostaglandin in response to either the ionophore (A23187) stimulation of divalent cation transport or the H-1 receptor stimulation with histamine.

Preliminary results have revealed alterations in the concentrations of basal prostaglandin and thromboxane in parenchymal and bronchial airway tissues of the lung within 30 minutes of total-body exposure of guinea pigs to 300 rads neutron-enriched radiation. At 30 minutes to 4 days postirradiation, parenchymal tissue levels of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), prostaglandin E (PGE), and thromboxane B_2 (TxB_2) were depressed overall, whereas airway tissues demonstrated elevated $PGF_{2\alpha}$ and TxB_2 generation at 1 and 3 hours, respectively. The effects of 300 rads neutron-enriched radiation on the capacity of parenchymal lung tissues to generate prostaglandin in response to ionophore provocation or histamine stimulation revealed increased sensitivity (enhanced synthesis) at 30 minutes and 4 days postirradiation. Airway lung tissues also demonstrated enhanced prostaglandin formation with ionophore challenge at 30 minutes, whereas histamine stimulation of these tissues did not evoke significant levels of PGE or TxB_2 at 30 minutes postirradiation.

The results suggest that significant alterations in the concentrations of prostaglandin and thromboxane in parenchymal and bronchial airway lung tissues do occur following neutron-enriched radiation. The time course and magnitude of these alterations differ from those observed in the lung tissues of animals that received

^{60}Co radiation. Similarly, the altered responsiveness to H-1 receptor stimulation and divalent cation transport that occurred after neutron-enriched radiation also deviated from the alterations seen following ^{60}Co irradiation.

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EFFECT OF GAMMA RADIATION AND THE RADIOPROTECTANT WR-2721 ON DEPIGMENTATION IN MOUSE HAIR

Principal Investigators: L. May, J. F. Weiss, V. Srinivasan, and G. N. Catravas

The effect of gamma radiation and WR-2721 on the depigmentation of mouse hair was studied by visual and electron paramagnetic resonance (EPR) measurements.

Male CD2F1 mice (10-12 weeks old) were maintained on a stock diet, with free access to water. They were exposed to various radiation doses (400-900 rads) with a cobalt-60 source, given at the rate of 40 rads per minute. Experimental animals were injected intraperitoneally with either 100 or 200 mg WR-2721 per kg body weight in phosphate-buffered saline (pH 7.4) 30 minutes before irradiation. Control animals were injected with an equivalent amount of phosphate-buffered saline. Hair samples were removed (with a pair of scissors) from the depigmented and the control areas of the animals, after sacrifice by asphyxiation with carbon dioxide.

The EPR measurements were made on samples of acetone-washed, air-dried hair. These hair samples were placed in quartz tubes, 12 mm x 4 mm outer diameter, which were marked so that the samples would always be situated in the same position in the EPR cavity. The sample tubes were open at both ends to facilitate loading and unloading of the hair using a 6-foot-long cotton-tipped applicator. These tubes were positioned in another quartz tube to ensure that the hair sample was always situated in the same position in the EPR cavity. The EPR signal was measured with a Varian model E-109 EPR spectrometer at room temperature.

Depigmentation as measured by the graying of the hair occurred after whole-body irradiation in patches, the nature and extent of which varied with the radiation dose. The injection of WR-2721 in non-irradiated mice did not cause depigmentation. The EPR data, which are related to the melanin content, indicate that in the mice that received the drug preirradiation, the hair in the depigmented area had higher EPR signals than did the hair from mice that had been only irradiated.

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EFFECT OF INDOMETHACIN ON LYSOSOMAL ENZYME ACTIVITY, CYCLIC NUCLEOTIDES, AND PROSTAGLANDINS IN IRRADIATED RATS

Principal Investigators: P. J. Trocha and G. N. Catravas

Recent experiments in our laboratory have shown that ionizing radiation causes an increase in the levels of prostaglandin and cyclic adenosine-3',5'-monophosphate, fluctuations in the concentration of cyclic guanosine monophosphate (cGMP), and leakage of enzymes from lysosomes. These studies suggest that prostaglandins, cyclic nucleotides, and lysosomes are involved with the inflammatory reaction resulting from radiation injury. Therefore, this investigation was performed with an in vivo system to determine if a mechanism could be obtained to explain how cyclic nucleotides, prostaglandins, and lysosomal enzyme systems are affected by gamma radiation. The prostaglandin inhibitor indomethacin was used in this study to determine if prostaglandins affect lysosomal stability and levels of cyclic nucleotide.

Sprague-Dawley rats (weighing 100-150 mg) were used in these experiments. The animals to be irradiated were exposed to 100 rads of ^{60}Co at a dose rate of 500 rads per minute. Indomethacin was dissolved in 0.05 M Na_2CO_3 (2.5 mg/ml) and administered at a dose of 10 mg/kg weight.

Indomethacin has been found to inhibit the increase in levels of liver and spleen prostaglandin in rats exposed to 1000 rads of ^{60}Co radiation. Animals that had been administered indomethacin at 70-90 minutes before sacrifice displayed no increase in levels of liver prostaglandin $F_{2\alpha}$ at 1 hour to 10 days postirradiation, compared to irradiated controls. Spleens taken from indomethacin-treated animals also showed no increase in levels of prostaglandin $F_{2\alpha}$ at 1-9 hours postirradiation. However, small increases in prostaglandin levels were observed at 1-10 days postirradiation compared with controls. The significant stabilization of lysosomal membranes and the reduction of lysosomal β-glucuronidase activity were further observed in spleen tissues removed from irradiated rats given indomethacin at various postirradiation intervals. More normal levels of spleen cGMP were also observed in the spleens of irradiated animals treated with indomethacin from 3 to 10 days postirradiation.

The analysis of nonirradiated animals receiving indomethacin had no effect on altering the concentrations of prostaglandin, levels of cyclic nucleotide, or stability of lysosomal membrane in spleen and liver, compared with rat tissues from nonirradiated controls given placebos.

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SYNERGISTIC INCREASE IN LEVELS OF CYCLIC NUCLEOTIDES IN IRRADIATED RATS ADMINISTERED METHYL XANTHINE

Principal Investigators: P. J. Trocha and G. N. Catravas

Recent studies have shown that ionizing radiation alters the levels of cyclic nucleotides and prostaglandins (1,2) by varying amounts in different mammalian tissues. The mechanism responsible for the variation of these levels after exposure to radiation is not clearly understood. However, it is likely that exposure to ionizing radiation affects some of the components associated with the synthesis and/or the breakdown of cyclic nucleotides. Therefore, we studied the combined effects of radiation and the phosphodiesterase inhibitor methyl-isobutyl xanthine on the levels of cyclic nucleotides in the liver and spleen of rats, in order to determine if exposure of the animals to ionizing radiation alters the activity of adenyl and guanyl cyclase enzymes.

Sprague-Dawley rats, 6-7 weeks old, were used in these experiments. The animals were irradiated bilaterally with a midline dose of 1000 rads of ^{60}Co at 500 rads/minute. The methyl-isobutyl xanthine (MIX) solution was administered at a dose of 11.5 mg/kg of animal weight.

MIX and ionizing radiation were found to cause increased levels of cyclic nucleotide in liver and/or spleen tissues. In nonirradiated rats administered MIX at 20-30 minutes before their sacrifice, the levels of spleen and liver cyclic adenosine monophosphate (cAMP) increased by 1130 ± 160 and 390 ± 120 pmoles/g tissue, respectively. Levels of spleen cyclic guanosine monophosphate (cGMP) rose by 40 ± 18 pmoles/g tissue, and liver cGMP content increased by 3 ± 2 pmoles/g tissue when compared with nondrug-treated controls. With radiation treatment, rats had maximal increases of 2000 ± 215 pmoles/g tissue for spleen cAMP at 6 days postirradiation, 50 ± 25 pmoles/g tissue for spleen cGMP at 1 day postirradiation, and 250 ± 100 pmoles/g tissue for liver cAMP at 6 hours postirradiation. Liver cGMP levels were not altered in irradiated rats. However, when rats were given radiation treatment and then administered MIX, the spleen and liver cAMP levels increased by 4000 ± 750 and 750 ± 100 pmoles/g tissue, respectively, at 6 hours postirradiation. The spleen cGMP content rose by 175 ± 30 pmoles/g tissue at 1 day postirradiation above the irradiated nondrug-treated controls. Therefore, it is concluded that administration of MIX to irradiated animals causes a synergistic increase in the levels of spleen cAMP, spleen cGMP, and hepatic cAMP.

REFERENCES

1. Trocha, P. J., and Catravas, G. N. Variation in cyclic nucleotide levels and lysosomal enzyme activities in the irradiated rat. Radiation Research 83: 658-667, 1980.

2. Trocha, P. J., and Catravas, G. N. Prostaglandin levels and lysosomal enzyme activities in irradiated rats. International Journal of Radiation Biology 38: 503-511, 1980.

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CHARACTERIZATION OF BETA-ADRENERGIC RECEPTORS ON CELLULAR AND PERIGRANULAR MEMBRANES OF PERITONEAL MAST CELLS OF RAT

Principal Investigators: M. A. Donlon, G. N. Catravas, and
W. A. Hunt, *AFRRI*
M. A. Kaliner, *National Institute of Allergy and Infectious Diseases*

A number of pathophysiological effects that occur following radiation injury can be attributed to histamine release from mast cells. Understanding the biochemical events controlling mast cell degranulation is an essential step in developing selective inhibitors to prevent the radiation-induced histamine release.

The surfaces of a mast cell contain a number of receptors that are capable of regulating intracellular events. Histamine and other vasoactive substances are stored intracellularly in dense granules, surrounded by a perigranular membrane. During secretion, the perigranular membrane fuses with the plasma membrane, and the granular contents are released into the external environment. Although numerous studies have partly defined the various components of the mast cell granule, little information is available on the nature of the perigranular membrane.

We have characterized beta receptors on the surface membrane of mast cells as well as the perigranular membrane. The number of β-receptors was determined by the binding of ^{3}H-dihydroalprenolol (^{3}H-DHA) after incubation (4^{o}C) with cellular material, followed by rapid filtration. The filters were washed and their radioactivity determined by liquid scintillation spectrometry. Specific binding was the difference between the ^{3}H-DHA binding and that found in the presence of 10 μM D,L-propanolol. Each mast cell contains 120 x 10^3 high-affinity (10.6 ± 2.6 nM) binding sites. The perigranular membrane contains 34 binding sites per granule, with an affinity of 7.0 ± 0.45 nM. Table 1 shows that the highest specific binding was to the perigranular membrane fraction.

Table 1. Distribution of ^{3}H-DHA Binding in Granules Isolated From Peritoneal Mast Cells

	Specific Binding (fmol/mg protein)	Total Specific Binding in Fraction (fmol bound/fraction)
Intact Granule	14.7	43.0
Membrane-Free Granule	0.82	1.1
Perigranular Membrane	30.5	48.8

Total specific binding in each fraction was determined by multiplying specific binding (fmol/mg protein) by total amount of protein in the fraction. These data are a representative experiment of three separate determinations.

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EFFECT OF GAMMA RADIATION ON RAT KIDNEY FUNCTION

Principal Investigators: L. M. Amende, M. A. Donlon, and
G. N. Catravas
Technical Assistance: W. W. Wolfe and J. E. Egan

The volume of urine has been shown to increase in rats following total-body irradiation. A number of factors control the volume and concentration of urine, but the primary mechanism in the renal tubule is the active transport of salt out of the ascending limb of the loop of Henle in the medulla. Thus, radiation could increase urine volume by its effect on the salt transport and/or water reabsorption.

We examined the effect of radiation on the sodium pump, which is the primary ion-transporting mechanism in the kidney. The sodium pump can be studied biochemically as the activity of the Na,K-ATPase or the K-dependent p-nitrophenylphosphatase, depending on the substrate used.

Sprague-Dawley rats were acclimated to metabolic cages, and urine was collected at various times pre- and postirradiation. Rats were sacrificed at 3, 6, or 12 hours after irradiation. The Na-dependent, K-ATPase-dependent, and K-dependent phosphatase activities were determined on kidney medulla and cortex tissue. The urine volume increased significantly over the sham-irradiated controls at 3 and 6 hours after irradiation, with the maximum volume change occurring at 6 hours postirradiation. The Na and K concentrations were unaltered following radiation, but significant increases occurred in the amounts of Na and K excreted by 6 hours. No alterations in either enzyme activity were found in the kidney cortex after irradiation, but at 6 hours postirradiation, the values for the medullary Na-dependent, K-ATPase-dependent (Figure 1), and K-dependent phosphatase activities (which provide a measure of the Na pump activity) were significantly less than the control values.

Thus, 900 rads of gamma radiation were found to affect the kidney function by increasing the urine volume, increasing the secretion of Na and K, and decreasing the Na pump activity.

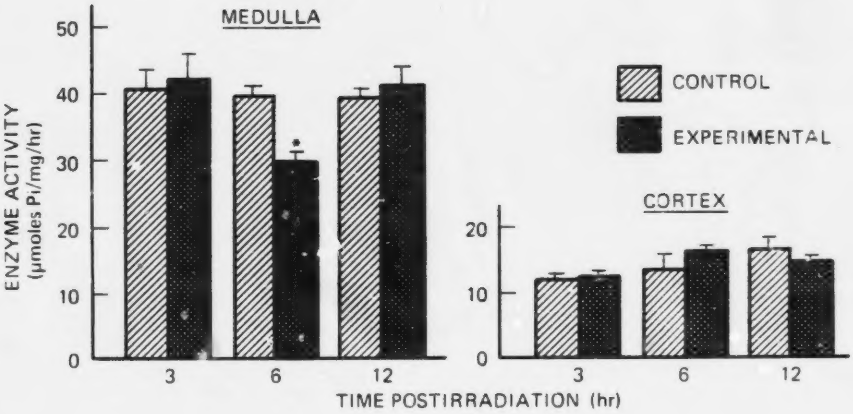

Figure 1. Effect of radiation on Na, K-ATPase activity. Enzyme activity was determined in both medulla and cortex at various times postirradiation. Note decrease in enzyme activity in medulla at 6 hr. * $p < 0.05$.

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EFFECTS OF WR-2721 ON RADIATION-INDUCED EXCRETION OF URINARY PROSTAGLANDIN

Principal Investigators: M. A. Donlon, L. K. Steel, E. A. Helgeson, and G. N. Catravas
Technical Assistance: A. Shipp and W. W. Wolfe

Previous studies have demonstrated that the radioprotective compound S-2-(3-aminopropylamine)-ethylphosphothionic acid (WR-2721) is concentrated in the kidney within 30 minutes after intraperitoneal injection of the drug. We have investigated the effects of WR-2721 on the excretion of urinary prostaglandin in ^{60}Co-irradiated rats.

Animals were housed in individual metabolic cages, fed a bolus of food daily, and allowed water ad libitum. WR-2721 (200 mg/kg) was dissolved in sterile Hank's balanced salt solution and injected intraperitoneally immediately after preparation (15-20 minutes before irradiation). Animals were irradiated with gamma radiation (from a ^{60}Co source) in individual lucite cages in groups of 10. Urine was collected, and the volume was measured at 3 days before irradiation and at various times after irradiation. The urine was analyzed for prostaglandins PGE and $PGF_{2\alpha}$ and for thromboxane B_2 (TxB_2) by radioimmunoassay.

The effects of WR-2721 on the levels of urinary PGE following gamma radiation are shown in Figure 1. WR-2721 administered before radiation caused significant reduction in PGE excretion at 3, 6, and 12 hours after 100 rads and at 1, 3, 6, and 12 hours after 300 rads with recovery. Dramatic reductions in radiation-induced PGE were observed after 900 rads, beginning at 6 hours postirradiation and continuing throughout the duration of the experiment (120 hours). Significant reduction was seen in the radiation-induced increases in urine volume in WR-2721-treated animals within the first 12 hours after irradiation.

Prostaglandins exhibit multiple actions on kidney function, including a role in the regulation of volume by counteracting the vasopressin action. These results suggest that WR-2721 may alter prostaglandin metabolism in vivo and thereby affect the urine volume. The decreases in prostaglandin excretion in WR-2721-treated animals may also reflect alterations in prostaglandin metabolism that are possibly related to the radioprotective effects of this drug in vivo.

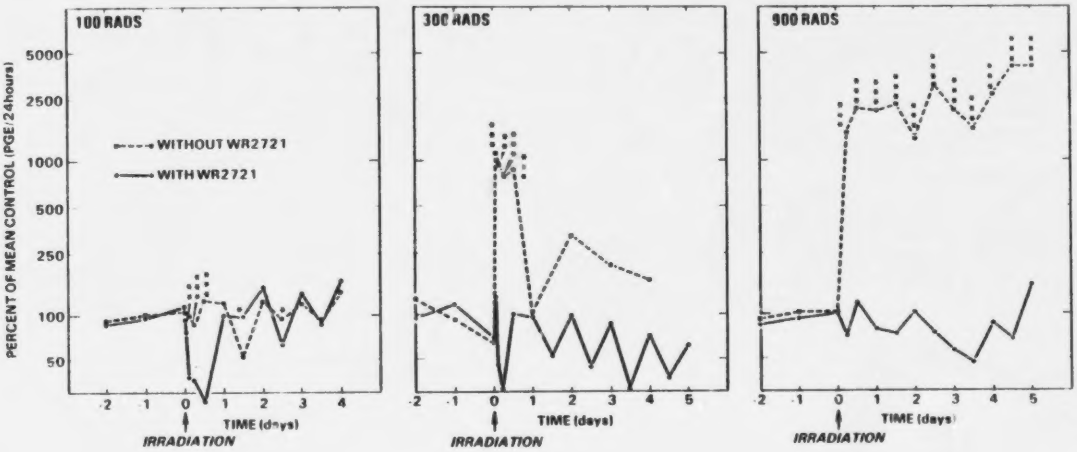

Figure 1. Values represent changes observed in urinary excretion (pg/24 hr) presented as geometric means (percent of mean control values) for animals irradiated with 100 rads (n = 10), 300 rads (n = 4), and 900 rads (n = 10) gamma radiation from a cobalt-60 source. Levels of significance are indicated as follows: * 0.05 ≥ p > 0.01; ** 0.01 ≥ p > 0.001; *** 0.001 ≥ p.

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ESTABLISHMENT OF THE HEMATOPOIESIS-INDUCING MICROENVIRONMENT IN THE MARROW OF MATRIX-INDUCED ENDOCHONDRAL BONE

Principal Investigators: K. F. McCarthy and M. Hale, *AFRRI*
S. Wientroub and A. H. Reddi, *National Institute of Dental Research*

The transplantation of demineralized bone matrix (DMB) from the rat femur to subcutaneous sites in allogeneic-recipient Long-Evans rats results in the formation of new endochondral bone, accompanied by the differentiation of hematopoietic bone marrow in the newly formed ossicle.

Briefly, mesenchymal cells appear in the implant on day 3; they proliferate and emerge as chondroblasts on days 5 and 6. On day 9, the onset of calcification of the matrix is evident. On day 10, vascular invasion of the calcified cartilage matrix occurs, resulting in chondrolysis and initial osteogenesis. The newly formed bone is remolded by osteoblasts, and bone marrow develops in the ossicle by day 21.

In the present study, we investigated the temporal relationship between the appearances of (a) the hematopoietic stem cells (CFU-S) and progenitor cells (GM-CFC) and (b) certain elements of the hematopoiesis-inducing microenvironment, i.e., fibroblast plaque-forming cells (P-CFC), colony-stimulating factor, and Type III collagen. Cells from the ossicles were harvested at specific time intervals by mechanical disruption of the ossicles, and they were assayed for CFU-S in irradiated A/J mice. GM-CFC were assayed in the double agar culture system using rat endotoxin-shocked lung-conditioned medium as a source of colony-stimulating factor.

CFU-S were first detected at 14 days postimplantation, and their numbers increased exponentially to day 24 (40 CFU-S per ossicle), decreased to 20 CFU-S per ossicle at day 30, and then increased once again to greater than 60 CFU-S per ossicle on day 36. Interestingly, P-CFC (scored as adherent colonies grown in 20% fetal calf serum Medium 199 for 20 days at 37°C and 5% CO_2) first appeared at day 10 (100 per ossicle), increased exponentially until day 15 (40,000 per ossicle), decreased until day 23 (900 per ossicle), and then once again slowly increased until day 35 (9000 per ossicle). A similar growth pattern was observed in the P-CFC compartments of the control femur marrow.

The ability of cells from the plaques or ossicles to synthesize colony-stimulating factor was tested by incubating one half an ossicle in 1 ml of minimum essential medium for 6 days at 37°C and 10% CO_2, and then testing the supernatant for colony-stimulating factor. This experiment failed to demonstrate a relationship between (a) the presence of colony-stimulating factor-secreting cells in the ossicles and (b) the appearance of CFU-S, GM-CFC, or P-CFC.

In summary, we found that growth curves for both ossicle CFU-S and ossicle P-CFC were bimodal, but out of phase. The P-CFC growth curve developed ahead of the CFU-S curve by 2-3 days. Further, the appearance of CFU-S did appear to correspond to a transition in collagen type from I to III within the ossicles at days 17 through 21. This work may indicate that (a) more than one type of P-CFC generates part of the hematopoiesis-inducing microenvironment and (b) some aspects of the regulation of P-CFC growth may be systemic in nature.

* * *

INFLUENCE OF RADIATION ON BONE DEVELOPMENT

Principal Investigators: J. F. Weiss and G. N. Catravas, *AFRRI*
S. Wientroub and A. H. Reddi, *National Institute of Dental Research*
Technical Assistance: L. V. Cummings and W. A. Rankin, *AFRRI*

The effects of radiation on bone development, remodeling, and healing of fractures have important clinical implications in orthopedic surgery, traumatology, and radiobiology, because radiation may retard the repair of the skeleton after combined injury (e.g., fracture in an irradiated subject). The basic characteristics of radiation injury to the skeleton have been studied for many years. Considerable evidence indicates diaphyseal and epiphyseal damage, which results in the retardation of bone growth. However, the cellular targets and underlying mechanisms involved are not yet clearly understood.

One of the difficulties in this research is the complexity of endochondral bone formation, with the entire sequence of bone formation occurring in a continuum in the epiphyseal growth plate. A method for studying the cell biology and biochemistry of endochondral bone formation has been developed.

The subcutaneous implantation of demineralized bone into allogeneic rats induces the formation of endochondral bone. In response to the implantation of matrix, a cascade of events ensues. It starts with the proliferation of mesenchymal cells at 3 days postimplantation, followed by chondrogenesis on day 7, calcification of the cartilagenous matrix on day 9, osteogenesis on day 11, and finally the formation of an ossicle containing active hematopoietic cells.

We investigated the effects of irradiation on the sequelae of the interaction of collagenous matrix and mesenchymal cells, cartilage differentiation, and bone differentiation (1). Anesthetized Long-Evans rats (1 month old) were irradiated in a vertical direction with a midline dose of 850 rads (cobalt-60, Theratron-80). Radiation entered the rats ventrally while a small area of the upper thorax was shielded. After irradiation, bone matrix was implanted in shielded and nonshielded sites, and transformation plaques were studied at various stages.

We found that on day 3, the incorporation of ^{3}H-thymidine (an index of cell proliferation) was inhibited by 70% in the nonshielded sites compared to the non-irradiated control rats. The degree of inhibition (35%) was less pronounced in shielded sites. Furthermore, recovery of cell proliferation was seen in the shielded sites, as opposed to the nonshielded contralateral sites. Similar differences were observed on day 7 as assessed by the incorporation of $^{35}SO_4$ into proteoglycans during chondrogenesis (Figure 1). Bone formation and mineralization were quantified on day 11 by alkaline phosphatase activity and ^{45}Ca incorporation. In nonshielded sites, a 73% inhibition of alkaline phosphatase activity occurred.

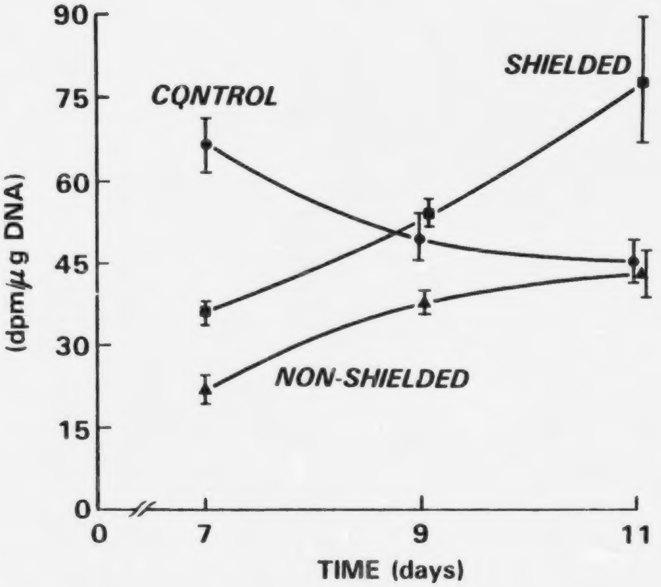

Figure 1. Changes in $^{35}SO_4$ incorporation into proteoglycans in implants in shielded and nonshielded sites in irradiated rats

In conclusion, radiation impaired progenitor cell proliferation, which resulted in decreased cartilage and bone differentiation. These findings imply that local mesenchymal cells respond to the implanted collagenous matrix.

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RADIATION-INDUCED IMMUNOSUPPRESSION AND THE EFFECT OF RADIOPROTECTIVE AGENTS AND BIOLOGIC RESPONSE MODIFIERS

Principal Investigators: J. F. Weiss and V. Srinivasan
Technical Assistance: W. A. Rankin and J. Jeng

Studies have continued on the postirradiation survival of mice and the mechanisms of radioprotection using various immune adjuvants (biologic response modifiers), in comparison to the standard radioprotective agent WR-2721. For example, the mechanism of radioprotection by azimexon (BM 12.531) is believed to increase in granulocytic and monocytic colony-forming cells after drug treatment (1).

The measurement of delayed-type hypersensitivity (DTH) by skin testing is a commonly used in vivo method in clinical and experimental immunology for determining alterations in cell-mediated immunity. A procedure for measuring DTH was developed (2) for use in our studies of radioprotection by immunomodulators and for determining the relation of immunoprotection to the increased postirradiation survival afforded by the more traditional radioprotective drugs.

The acquisition and expression of DTH can be suppressed by whole-body radiation. Radiation-induced suppression of DTH may be viewed mainly in terms of radiation damage to activated T-lymphocytes and/or macrophages, which are both required for eliciting DTH. The effect of radioprotectors on the radiation-induced suppression of cellular immunity as measured by the DTH response has not been studied in detail.

In the present studies, DTH was elicited with the contact allergen oxazolone (4-ethoxymethylene-2-phenyl oxazolone). CD2F1 male mice were sensitized with 4% oxazolone in acetone:olive oil on the shaven abdomen. After 4 days, sensitized animals were challenged with 1% oxazolone on the left ear, and carrier was applied on the right ear as a control.

Changes in ear thickness were measured after 24 and 48 hours with a micrometer. Animals were irradiated with cobalt-60 (40 rads/minute) at various times either before or after sensitization. Radiation (700 rads) suppressed the acquisition of the DTH response when sensitization followed irradiation. When animals were irradiated after sensitization, a dose-dependent reduction in DTH was observed (Figure 1). WR-2721 (100 and 200 mg/kg body wt, intraperitoneally) given 30 minutes before irradiation (700 rads) significantly reversed the suppression of DTH. The drug was also effective when administered on the day of sensitization with oxazolone (3).

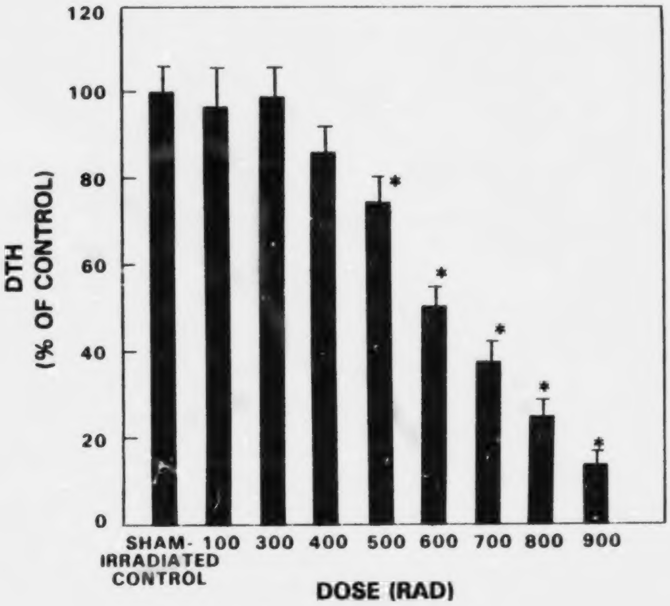

Figure 1. Radiation effect on expression of DTH to oxazolone in mice. Irradiation was at 2 days after sensitization with oxazolone, and challenge was 2 days after irradiation. Ear thickness (difference between ears after application of oxazolone and carrier, mean ± SE) was measured 24 hours after challenge. * Irradiated groups (10 mice/group) that differed significantly ($p < 0.001$) from sham-irradiated control group.

Further studies are in progress on the mechanism of protection and the effect of other radioprotective agents and immunomodulators on the DTH response after irradiation.

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VITAMIN E AND RADIATION INJURY

Principal Investigators:	V. Srinivasan, J. F. Weiss, A. J. Jacobs, and S. A. Simpson
Technical Assistance:	C. Morris, L. V. Cummings, and W. A. Rankin

Vitamin E is an integral part of the mammalian antioxidant defense system, and it appears to protect against various oxidative stresses, including chemicals, drugs, and ionizing radiation. There is evidence that vitamin E supplementation protects against radiation-induced lipid peroxidation, but few in vivo studies have been done on the role of vitamin E on radiation injury, other than on postirradiation survival.

An increase in survival of whole-body irradiated rodents treated with vitamin E has not been clearly established. Vitamin E may be beneficial in counteracting the impairment of immune function due to radiation and chemical exposure, since vitamin E has been shown to enhance the humoral immune response and lymphocyte stimulation in vitro.

In the present studies (1,2), the effect of vitamin E on radiation injury in mice was determined on (a) the 30-day survival after whole-body irradiation (a measure of hematopoietic system damage), (b) cell-mediated immunity as measured by delayed-type hypersensitivity (DTH) to oxazolone, and (c) alterations in the hepatic drug-metabolizing system and the glutathione peroxidase activity.

The postirradiation survival of CD2F1 male mice that had been fed a diet supplemented with three times the normal dietary level of vitamin E was enhanced at radiation doses of 750 and 850 rads. Whole-body irradiation at 300-900 rads resulted in a dose-dependent suppression of cellular immunity as measured by changes in ear thickness in mice sensitized (preirradiation) and challenged (postirradiation) with oxazolone. Dietary vitamin E treatment significantly reversed the radiation-induced immunosuppression as measured by the DTH response in mice irradiated with 700 rads. The DTH response in irradiated mice given injectable vitamin E preparations depended on the dose, vehicle, and treatment schedule with respect to irradiation (Figure 1). Vitamin E treatment also resulted in alterations in drug-metabolizing enzymes. Cytochrome P-450 and cytochrome b_5 were lower in vitamin E-treated irradiated mice than in irradiated control mice.

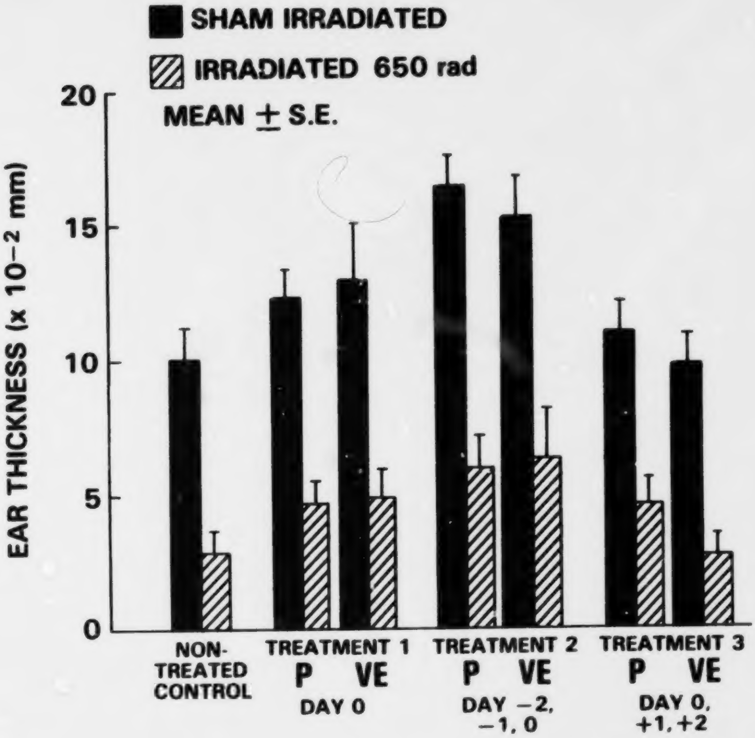

Figure 1. DTH to oxazolone in irradiated (650 rads) mice injected intraperitoneally with placebo (P) or vitamin E (VE) (50 mg/kg) at various times with respect to irradiation (day 0). Treatment 1: Single injections of Hoffman-La Roche preparations of P or VE at 1 hr before irradiation. Treatments 2 and 3: Injections of P or VE on days indicated, with day-0 injection given 1 hr before irradiation. Values are mean differences in ear thickness at 24 hr after challenge with oxazolone for groups of six mice.

Cytosol glutathione peroxidase activity showed a reduction after irradiation, which was reversed by the administration of vitamin E.

The protective effect of vitamin E on the post-irradiation survival, immunological competence, and biochemical changes at the tissue level was demonstrated in the present studies.

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ISOLATION OF HEMATOPOIETIC STEM CELL

Principal Investigator: K. F. McCarthy

Murine hematopoietic stem cells were purified 200-fold using a combination of density gradient centrifugation and fluorescence-activated cell sorting (FACS-II).

The first step involved centrifuging the cells through an albumin gradient, and collecting those cells with a buoyant density of less than 1.092 gm/cm^3. These cells were tagged with rhodomine wheat-germ agglutinin and fluorescein isothiocyanate-labeled anti-H-2K^k, and sorted on the dual laser FACS-II.

Although significant enrichment could be achieved, the yield of cells was very low. Efforts were made to scale up the procedure, using affinity columns and cell "panning."

* * *

EFFECT OF MOUSE TYPE-I INTERFERON ON MOUSE BONE MARROW CELLS AND PERITONEAL EXUDATE CELLS CULTURED *IN VITRO*

Principal Investigators: M. L. Hale and K. F. McCarthy

Type-I mouse interferon significantly reduces the macrophage colony formation from mouse bone marrow cells or mouse peritoneal exudate cells, when cultured in vitro in the presence of colony-stimulating factors (CSF) derived from either pregnant mouse uterus ($\mathrm{CSF}_{\mathrm{PMUE}}$) or concanavalin-A-purified preparation from endotoxin-shocked mouse lung-conditioned medium ($\mathrm{CSF}_{\mathrm{LCM}}$).

The effect of interferon on granulocyte (G-CFU) colonies or granulocyte-macrophage-mixed (GM-CFU) colonies depended on the colony-stimulating factor used. G-CFU and GM-CFU were somewhat elevated in interferon-treated cells stimulated by $\mathrm{CSF}_{\mathrm{PMUE}}$ whereas G-CFU and GM-CFU were slightly inhibited in interferon-treated cells stimulated by $\mathrm{CSF}_{\mathrm{LCM}}$. The most dramatic effect of interferon was observed in the macrophage population.

We conclude that interferon affects differentiation preferentially at the level of the macrophage progenitor cell.

* * *

DETERMINATION OF AUTHENTIC HISTAMINE IN RAT URINE

Principal Investigators: M. A. Donlon, E. A. Helgeson, and
G. N. Catravas
Technical Assistance: W. W. Wolfe and K. Lambright

It is well established that histamine is released from mast cells as a result of midlethal and lethal doses of radiation in humans (1,2) and in a variety of animals (3,4). The physiological significance of the mast cell has not been fully appreciated because of its widespread distribution throughout the body. But the total number of mast cells in the body has been calculated as equivalent to the size of the spleen (5). Plasma histamine, which circulates through the kidney, is excreted intact into the urine. Therefore, measurement of histamine in the urine of laboratory animals can be used to monitor fluctuations in plasma histamine that occur in response to radiation. The widespread distribution of the mast cells in the body, coupled with the stability of histamine in the urine, presents ideal possibilities for monitoring the urinary histamine as a biological dosimeter.

This study reports the techniques we have developed to determine the levels of authentic histamine in rat urine. A diagram of the current methodology for assaying histamine in rat urine is shown in Figure 1. An essential component of this assay sysem is the incubation with diamine oxidase (DAO) of the urine sample at pH 7.0. Two results are obtained for each specimen. The sample treated with DAO, which metabolizes endogenous histamine, represents histamine fluorescence. The sample not exposed to DAO represents nonhistamine fluorescence. The difference between these two samples represents that portion of fluorescence that may be attributed to authentic histamine. Results can be expressed either as ng/ml or ng/24 hours, when corrected for urine volume. A dose response for DAO metabolism of histamine in rat urine is shown in Figure 2. All urine samples are routinely incubated with 7.5 U/ml DAO, a concentration (of DAO) that metabolizes 100% of urinary histamine.

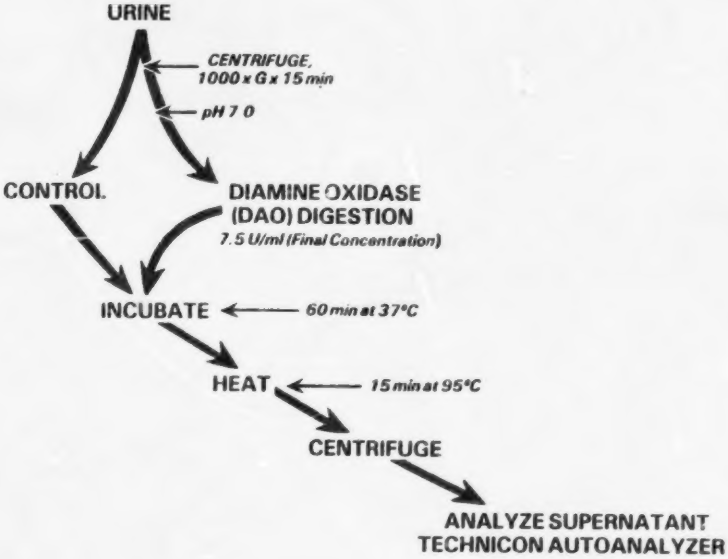
URINE
CENTRIFUGE, 1000 x G x 15 min
pH 7 0
CONTROL
DIAMINE OXIDASE (DAO) DIGESTION
7.5 U/ml (Final Concentration)
INCUBATE
60 min at 37°C
HEAT
15 min at 95°C
CENTRIFUGE
ANALYZE SUPERNATANT TECHNICON AUTOANALYZER

Figure 1. Flow diagram for determination of histamine in rat urine. In order to determine authentic histamine content in rat urine, each sample was treated with diamine oxidase. Authentic histamine was digested, allowing subtraction of nonhistamine-associated fluorescence from total fluorescence.

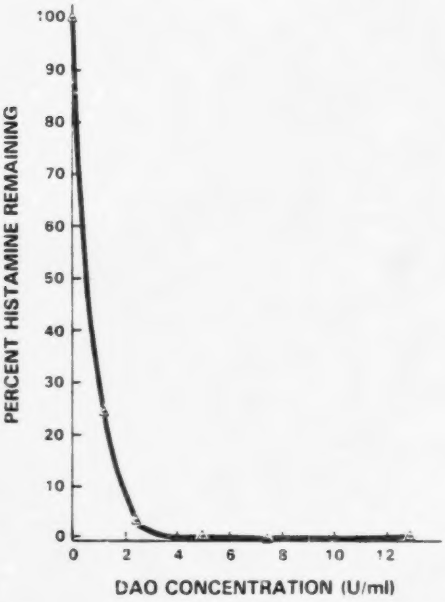

Figure 2. Dose response of diamine oxidase (rat urine). Histamine (500 ng/ml) was added to 10 ml of rat urine, pH 7.0, and incubated at 37°C for 60 min with increasing concentrations of DAO. Reaction was terminated by heating for 15 min at 95°C. Supernatant was assayed for histamine by an automated fluorometric assay.

Authentic histamine values for Sprague-Dawley rats were found to be 583.17 ± 104 ng/ml (n = 20) for male rats and 2018.40 ± 182.09 ng/ml (n = 20) for female rats. Female rats excrete about four times as much histamine as male rats, with less variation. The effect of total-body radiation on histaminuria in male and female rats is being evaluated.

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EFFECTS OF RADIATION AND OTHER FACTORS ON THE MAMMALIAN BRAIN

Principal Investigators: G. N. Catravas, J. P. Christopher, M. Tavens, and J. Boose

A description of the interaction between neural components and ionizing radiation is important in understanding the underlying factors that influence an individual's ability to react and recover after exposure to radiation.

Preliminary studies were performed on the neural enzyme acetylcholinesterase, which functions to remove the mediator acetylcholine from the synapse in order to allow repolarization to occur. The enzyme from the electric eel, Electrophorus electricus (the richest known source), was studied in varying stages of purity.

Initial results indicate that the enzyme was considerably more stable as a homogeneous protein in its in vivo environment than it was in vitro (Table 1). In fact, the enzyme in irradiated whole tissue retained 83% of its initial activity at 200,000 rads, whereas pure acetylcholinesterase exhibited a 27% loss of activity at only 250 rads. These results are consistent with the kinetics of inactivation in the presence of additional substances, i.e., other reactive biological components (1).

Table 1. Radiosensitivity of Acetylcholinesterase at 100,000 Rads Cobalt-60 as a Function of Its Purity

Sample	% Activity Remaining
Whole tissue	87
Crude homogenate	97
Homogeneous enzyme	0

Parallel studies on the structural integrity and functional integrity of acetylcholinesterase upon exposure indicated that the pure enzyme was very radiosensitive, fragmenting into two pieces of unequal molecular weight. Analysis for tryptophan content revealed that this amino acid is destroyed, as is the case for photodestruction of enzyme (2). Current studies focus on (a) describing in detail the enzyme inactivation, and (b) finding ways to render the protein more radioresistant.

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EXPERIMENTAL HEMATOLOGY DEPARTMENT

The Experimental Hematology Department investigates the effects of ionizing radiation and other militarily relevant stressors on the hematopoietic system. Emphasis is centered within three major categories of the Biomedical Effects Research Program: (a) the prevention and treatment of radiation effects, including radiation-induced hematopoietic dysfunction, medical and surgical therapy of combined effects, and radioprotectants; (b) the biomedical effects of fast neutrons; and (c) a technology base for experimental hematology and cellular radiobiology. Areas of research within these categories are organized within the framework of three Divisions in the Department: Hematology, Immunology, and Cellular Radiobiology.

The main objective of the Hematology Division is to delineate the mechanisms involved in regulation of the processes of proliferation and differentiation of the hematopoietic stem cell and its committed progeny in both granulocyte-macrophage and erythroid cell lines. Areas of research include

Enhancement of postirradiation recovery in stem and progenitor cell populations

Animal models for study of infectious disease and its effect on the hematopoietic system in normal hosts and irradiated hosts

Stem cell physiology, cellular regulation, and humoral regulation in the murine, canine, and monkey model systems

Early and late effects of gamma and neutron irradiation

Cellular and humoral hematologic and immunologic consequences of combined effects of ionizing radiation and traumatic injuries, and

Transplantation of bone marrow and peripheral blood cellular fractions that are capable of hematopoietic restoration in lethally irradiated large-animal species, such as the dog and monkey.

Experimental programs within the Immunology Division are aimed at the mitigation of gamma and neutron radiation-induced lymphomyelopoietic aplasia and its attendant cellular and humoral immune dysfunctions. Specific objectives include

Novel applications of radioprotective agents

Transplantation of bone marrow cells, and techniques to prevent graft-versus-host disease in the murine system

Contributions of host intestinal microflora in irradiated animals

Humoral control of the cellular immune and myelopoietic systems

Lymphomyelopoietic alterations and mechanisms of action of wound trauma either alone or with radiation injury, and

Cellular and humoral immune responses in the acute-phase reaction to inflammation and tissue injury in normal animals and irradiated animals.

The main objectives within the Cellular Radiobiology Division are to delineate the control of hemopoietic stem cell proliferation after exposure to ionizing radiation and to locate, identify, and quantitate radiation-induced or drug-induced DNA damage and to subsequently bring about its repair. Areas of research include

Investigation of the relationship between cell proliferation and repair of molecular lesions produced by ionizing radiation

Determination of the molecular basis of synergism between ionizing radiation and chemical agents

Development of methodology for enhancing the proliferation of bone marrow stem cells post-irradiation, and

Investigation of, through biochemical and molecular approaches, the relationship between DNA damage and cell proliferation, including the chemical isolation of particular DNA lesions and the quantitation of its repair.

Attaining the research objectives or milestones within these projects will also add considerable information to fulfilling the military research requirements as recently published by the Army. They are the response of combat troops to nuclear radiation, the incidence of casualties from combined effects, and the biomedical effects of ionizing radiation. Specific requirements that have been identified include

Amount of biological repair (recovery) that occurs following exposure to nuclear radiation

Biological effects of cumulative exposure

Neutron relative biological effectiveness

Reliability of the extrapolation of large-animal models to man

Biological effects of combined injury, including the simultaneous insults and the sequential insults that the soldier may receive along with nuclear weapons effects, such as those associated with conventional and chemical munitions and the later development of sepsis, and

Mechanisms of biological damage to sensitive cells and the critical targets within them.

* * *

HEMATOPOIETIC RESPONSE OF SPLENECTOMIZED C3HeB/FeJ AND C3H/HeJ MICE TO LIPOPOLYSACCHARIDE

Principal Investigator: T. J. MacVittie
Associate Investigator: S. R. Weinberg
Technical Assistance: J. L. Atkinson, D. P. Dodgen, and
R. T. Brandenburg

The paired inbred mouse strains C3HeB/FeJ and C3H/HeJ differ in their extramedullary hemopoietic responses to lipopolysaccharide (LPS). The C3H/HeJ mice exhibit reduced responses relative to the C3HeB/FeJ mice, in terms of colony-stimulating factor, endogenous and exogenous spleen-derived stem cells (CFU-s), and spleen-derived granulocyte-macrophage colony-forming cells (GM-CFC), whereas equivocal responses were noted for the marrow-derived CFU-s and GM-CFC.

In an effort to determine if the mutational defect was specifically limited to extramedullary expression, we studied C3HeB/FeJ mice and C3H/HeJ mice at 6 weeks postsplenectomy. The splenectomy emphasizes the medullary response to an intraperitoneal injection of 10 µg Eschericnia coli (LPS-W).

Significant differences were revealed in the responses of C3HeB/FeJ and C3H/HeJ marrow-derived cells. Total cells [CFU-s, GM-CFC, and the macrophage colony-forming cell (M-CFC)] in the C3HeB/FeJ strain all decreased significantly from their saline-injected control values as well as from their counterpart C3H/HeJ values within 48 hours after injection of LPS-W. The decline in C3HeB/FeJ CFU-s, GM-CFC, and M-CFC values was followed by an overshoot, with subsequent return to control values (Figure 1). The total nucleated cells (CFU-s, GM-CFC, and M-CFC) derived from the C3H/HeJ marrow were characteristically nonresponsive, and never varied significantly from their saline-injected control values over the observation period.

These results suggest that the phenotypic effect of the defective gene can extend to all hemopoietic tissue, medullary and extramedullary, that normally responds to the factors released through the genetic response to lipopolysaccharide.

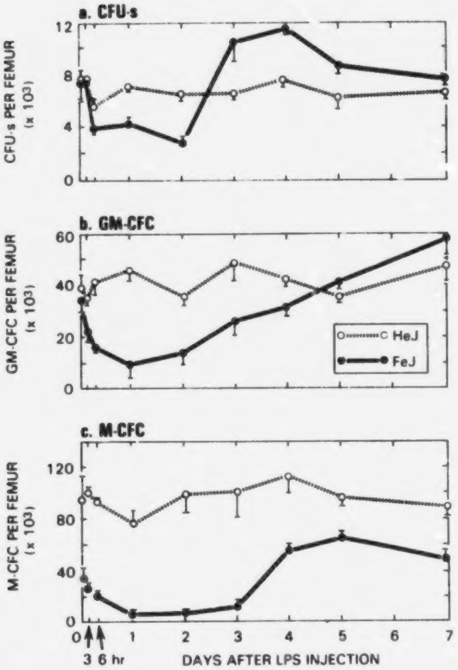

Figure 1 a,b,c. Number of (a) CFU-s, (b) GM-CFC, and (c) M-CFC per femur in splenectomized C3HeB/FeJ and C3H/HeJ mice at various times after i.p. injection of 10 μg *E. coli* LPS-W. Values are means (± SEM) of five replicate experiments.

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HEMATOPOIETIC RESPONSE TO 150 RADS COBALT-60 GAMMA AND MIXED NEUTRON-GAMMA RADIATION IN A CANINE MODEL OF COMBINED INJURY

Principal Investigators: T. J. MacVittie, D. F. Gruber, M. L. Patchen,
L. M. Wathen, R. Monroy, and J. J. Conklin,
AFRRI
J. E. French and G. Murano, *Food and Drug Administration*
M. Smith, L. Casey, and R. I. Walker,
Naval Medical Research Institute

The Problem

The nuclear battlefield will present a new challenge to the military surgeon. Most casualties will result from a combination of radiation and trauma. Either radiation or trauma under battlefield conditions is a problem in itself, but the two combined can transform the single, sublethal injury into a lethal one. The decrease in survival with combined injuries has been observed for many years with victims of automobile accidents. Blunt or sharp trauma by itself may cause minimal morbidity, but the addition of a small burn often results in death. Injury from radiation can greatly magnify the problem.

Experiments in animals have shown that the addition of sublethal irradiation to an existing burn with an area of 20% body surface will raise the mortality rate from 12% to 73%. Based on the Hiroshima and Nagasaki experiences, the estimated incidence of combined injuries is as follows: for burn, wound, and radiation, 20%; for burn and radiation, 40%; for mechanical injury and radiation, 5%; and for others, 35%.

Based on these and other relevant experimental data, it is imperative that we develop reliable animal models for investigating the pathophysiological effects of combined injuries and their consequent medical and surgical treatments.

AFRRI Combined-Injury Project

AFRRI supports the development of a collaborative research project investigating the pathophysiology of combined injury. The following outlines the parameters and various models being investigated:

A. Animal Model: Mouse

1. Skin wound
2. Colon resection and anastomosis
3. Sepsis

B. Major Variables

1. Radiation dose, quality (cobalt-60, neutron)
2. Regimen (acute, fractionated, whole-body, partial-body)
3. Trauma, sepsis, timing of variables

C. Parameters

1. Hematological
2. Immunological
3. Serum factors
4. Acute-phase reactants
5. Coagulation
6. Prostaglandins
7. Sepsis
8. Wound healing
9. Therapy
10. Others

The following summarizes the current results on the hematopoietic response to combined injury in the canine model being established at AFRRI. These data concern

- Dose response of granulocyte-macrophage colony-forming cells (GM-CFC) to cobalt-60 and mixed neutron-gamma radiation

- Hematopoietic response in the dog to 150 rads of midline total-body exposure of cobalt-60 gamma and mixed neutron-gamma radiation

- Effect of combined injury on recovery of the hematopoietic system to 150 rads of gamma irradiation.

In these studies, trauma is presented as surgical splenectomy performed within 1-2 hours after irradiation.

Results

1. The dose-response curves of marrow-derived GM-CFC to cobalt-60 radiation and to mixed 0.8-MeV neutron-gamma radiation are significantly different, with D_0 values of approximately 73 rads and 30 rads, respectively. The relative biological effectiveness based on midline tissue doses would be approximately 2.4. The mixed neutron-gamma ratio is 6:1 at the skin, and it dissipates to approximately 1:1 at midline.

2. In Table 1 are the nadirs, with respect to the time and percent of control values and the time required for recovery of white blood cells, platelets, and GM-CFC for the peripheral blood leukocytes (PBL) and bone marrow (BM) after 150 rads of cobalt-60 gamma radiation. Irradiation with the mixed neutron-gamma field results in a significant relative biological effect, as evidenced by greater depression in values after exposure and delayed recovery time. It may take as long as 42 days for marrow-derived GM-CFC to return to within normal concentration values, compared to the 28 days noted after 150 rads.

Table 1. Hemopoietic Response to 150 Rads Cobalt-60 Whole-Body Irradiation

	Nadir, % Control	100% Recovery
White blood cells	7 days, 28	49 days
Platelets	10 days, 25	35 days
PBL GM-CFC	1 day, 0	42 days
BM GM-CFC	1 day, 9	28 days

3. Splenectomy (SX) of normal dogs resulted in significantly elevated levels of peripheral blood leukocytes and platelets through 24 days postsurgery. A similar effect of splenectomy is seen in the irradiated dog relative to the irradiated control; that is, the

peripheral blood leukocyte and platelet values in the 150-rads-irradiated-SX dogs are consistently higher at every time point than in the 150-rads-irradiated only dogs. This apparent advantage is not carried over to the recovery of the marrow- and peripheral blood-derived granulocyte-macrophage progenitor cells. The recovery of relative numbers (or the concentration of GM-CFC from these sites) in the 150-rads-irradiated dogs occurs earlier (day 21-28) than in the 150-rads-irradiated-SX dogs (day 28-35). The advantage in recovery of peripheral leukocytes is apparent because we have not performed functional assays with those granulocytes, lymphocytes, or monocytes. They may represent effete cells that would have been cleared by a functioning spleen.

Current and Future Directions of Research

Replicates of these splenectomy studies are being conducted to establish a data base with a number of the other parameters previously mentioned. These include (a) acute-phase reactants such as C-reactive protein, fibrinogen, and α-1 glycoprotein; (b) prostaglandin; (c) various aspects of the coagulation scheme such as platelet factors and protease inhibitors; (d) various functional assays for monocytes and granulocytes such as phagocytosis and microbicidal ability; and (e) immunological parameters, including T and B cells and release of lymphokines.

The major variables are also being manipulated. The next choices of trauma are colon resection and hemorrhagic shock. The initial choice of concurrent 150 rads total-body irradiation will be maintained. Manipulation of the radiation has involved delineating the response to 150 rads mixed neutron-gamma. This will be included with the resection trauma. The time variable will also be changed so that irradiation will precede trauma by approximately 3-5 days. All surgical procedures have been performed under sterile conditions, and will continue so. Bacterial sepsis will be added to the radiation-trauma scheme at a future date. A canine sepsis model is being developed.

◆

EFFECTS OF ENDOTOXIN ON SURVIVAL OF HYPERTRANSFUSED MICE

Principal Investigators: R. M. Vigneulle and S. J. Baum
Technical Assistance: R. T. Brandenburg

The primary problem for a mammal that receives whole-body exposure to a midlethal dose of gamma radiation is hemopoietic dysfunction. This is manifested in the diminished capacity of bone marrow to provide the necessary granulocytes to resist infections, during the critical first few weeks after irradiation when survival is determined.

We investigated ways to enhance granulopoiesis in the irradiated animal as an objective of the AFRRI task 3D, concerned with radiation and hemopoietic dysfunction. A hypertransfused B6CBF1 female mouse was selected as the model to test the following hypothesis: Endotoxin, a lipopolysaccharide derivative of bacterial cell walls, stimulates granulocytopoiesis through increased progenitor cell proliferation, which may improve survival when the demand for erythropoiesis is suppressed.

Normal mice or hypertransfused mice received 8.5, 9.0, or 9.5 grays of cobalt-60 gamma rays in a bilateral exposure mode, at a dose rate of 0.4 gray per minute in the AFRRI cobalt-60 gamma source facility. Immediately following irradiation, groups of control mice and hypertransfused mice received either an intraperitoneal injection of 10 μg endotoxin S. typhimurium or pyrogen-free saline. They were placed into cages (five mice per cage) and observed for deaths until day 40.

Figure 1 shows the survival data for the four treatment groups through day 40. Endotoxin alters the acute survival pattern of the group of hypertransfused mice (combined treatment) exposed to these midlethal doses of cobalt-60 gamma rays. Survival of the combined-treatment group depended on the radiation dose. The most pronounced effect on survival of the combined-treatment groups followed the 9.0-gray gamma dose. At this dose, survival of the combined-treatment group was significantly different ($0.01 > p > .001$) from that of irradiated mice given saline, irradiated mice given endotoxin, and hypertransfused mice that were irradiated and given saline.

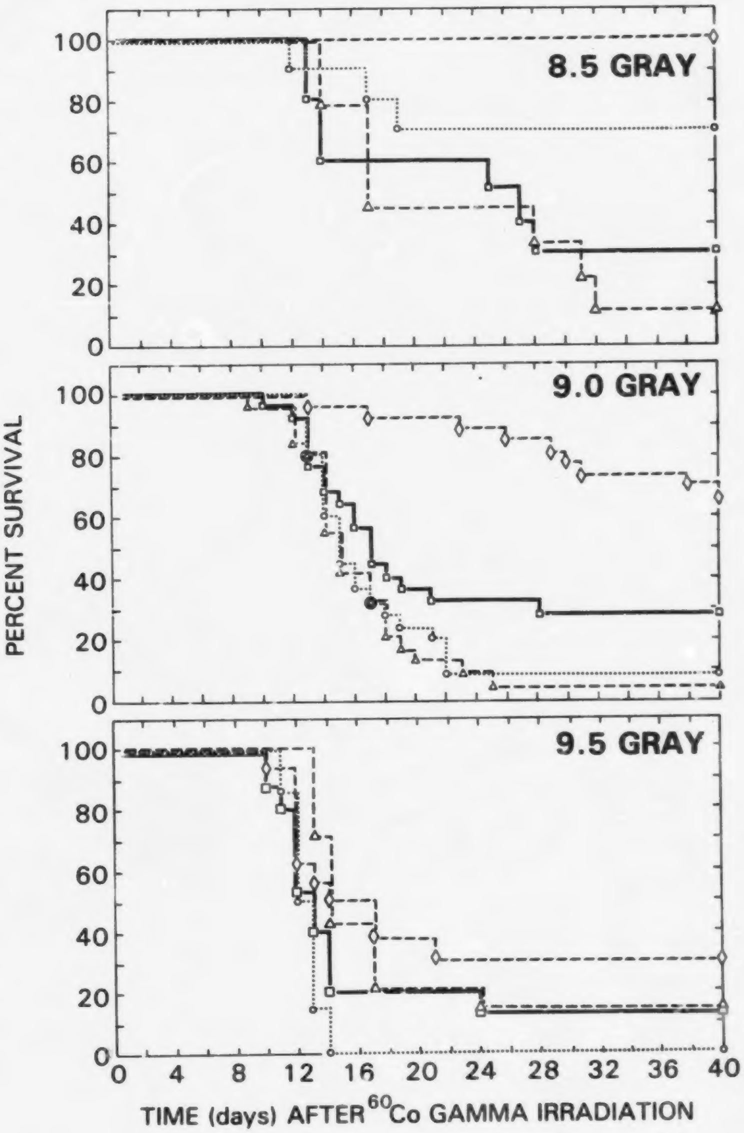

Figure 1. Survival versus time (days) after cobalt-60 gamma irradiation for female mice that received these four treatments: irradiation + saline, open circles; irradiation + 10 μg endotoxin, open squares; hypertransfusion + irradiation + saline, open triangles; hypertransfusion + irradiation + 10 μg endotoxin, open diamonds.

These data from the combined-treatment protocol show that endotoxin is much more effective in enhancing survival postirradiation when the demand for erythropoiesis is suppressed. Hypertransfused mice have greatly expanded pools of uncommitted progenitor and myeloid precursor cells which apparently are unstimulated. When these pools were stimulated by endotoxin after irradiation, granulocytopoiesis was enhanced, resulting in increased animal survival (1).

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EFFECTS OF A SOLUBLE GLUCAN ON PROLIFERATION OF HEMOPOIETIC STEM CELLS

Principal Investigator:	M. L. Patchen
Associate Investigator:	T. J. MacVittie
Technical Assistance:	J. L. Atkinson and B. Watkins

Particulate glucan (glucan-P), obtained from the inner cell wall of the yeast Saccharomyces cerevisiae, has been shown to significantly alter the proliferation of pluripotent hemopoietic stem cells (CFU-S), granulocyte-macrophage colony-forming cells (GM-CFC), and erythroid colony-forming cells (CFU-e) in murine bone marrow (BM) and spleen (SPL) (1-3). However, the practical use of particulate glucan is limited, because of its "occlusion-type" neurovascular side effects after large doses of intravenously administered glucan-P. Particulate glucan was solubilized recently, and we report here the hemopoietic effects of one such soluble glucan (glucan-C).

Female C3H/HeN mice were intravenously injected with 1.5 mg of glucan-C. At 2, 5, and 13 days later, the bone marrow and splenic cellularity, CFU-S, GM-CFC, and CFU-e were assayed (Table 1). Compared to control values, the bone marrow cellularity increased by 25% on day 13, and splenic cellularity increased by 30% and 100% on days 5 and 13. Bone marrow CFU-s and CFU-e were not altered after administration of glucan-C. However, splenic CFU-s increased by 133% and 433% on days 5 and 13, and CFU-e increased by 225%, 250%, and 350% on days 2, 5, and 13, respectively. After glucan-C treatment, the bone marrow GM-CFC increased by 72% on day 13, and the splenic GM-CFC increased by 169%, 200%, and 352% on days 2, 5, and 13, respectively.

Table 1. Hemopoietic Effects Produced by Intravenous Administration of Soluble Glucan-C

Day After Glucan-C Treatment	CFU-s per		GM-CFC per		CFU-e per	
	Femur (x10^3)	Spleen (x10^4)	Femur (x10^4)	Spleen (x10^4)	Femur (x10^4)	Spleen (x10^6)
0 (control)	2.0±0.1	0.6±0.1	2.1±0.1	1.3±0.1	4.1±0.2	0.4±0.1
2	1.7±0.2	0.7±0.2	2.0±0.2	3.5±0.4	3.1±0.6	1.3±0.2
5	1.8+0.3	1.5±0.3	2.2±0.3	3.9±0.4	3.9±0.5	1.4±0.2
13	2.2±0.2	3.2±0.4	3.9±0.4	6.4±0.7	4.9±0.6	1.8±0.3

We conclude that this soluble glucan can also function as a potent hemopoietic modulator.

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ENHANCEMENT OF HEMOPOIETIC RECOVERY AFTER EXPOSURE TO COBALT-60 IRRADIATION BY THE IMMUNOMODULATING AGENT GLUCAN

Principal Investigator: M. L. Patchen
Associate Investigator: T. J. MacVittie
Technical Assistance: G. A. Davis, J. L. Atkinson, and B. Watkins

The proliferation of murine bone marrow and splenic pluripotent hemopoietic stem cells (CFU-s), granulocyte-macrophage colony-forming cells (GM-CFC), and erythroid burst- and colony-forming cells (BFU-e, CFU-e) has been shown to be altered after administration of the particulate form of the immunomodulating agent glucan (1-3). Because of glucan's ability to modulate hemopoiesis, we examined the feasibility of using this agent to enhance hemopoietic recovery following exposure to a hemopoietically damaging dose of cobalt-60 irradiation.

Female C3H/HeN mice received a single intravenous injection of either 0.4 or 1.5 mg of particulate glucan at times ranging from 1 hour to 17 days either before or after exposure to 650 rads of cobalt-60 irradiation. Eight days later, mice were sacrificed, the spleens were removed, and the number of endogenous hemopoietic spleen colony-forming units (E-CFU) were counted. The effects of glucan were dose-dependent, with greater and more prolonged effects observed after the treatment with 1.5 mg of glucan (Table 1). The E-CFU numbers increased with both glucan doses, injected at all times before irradiation.

Table 1. Effect of Pre- and Postirradiation Glucan Treatment on E-CFU*

	PREIRRADIATION					POSTIRRADIATION				
Time of Glucan Injection (Days)	-17	-11	-5	-1	-1 hour	+1 hour	+1	+5	+11	+17
	Resulting E-CFU (%)†					Resulting E-CFU (%)†				
Amount of Glucan Injected (Mg)										
0.4	174	261	306	539	548	590	408	141	‡	‡
1.5	326	442	990	1448	1503	1355	447	260	‡	‡

* E-CFU, endogenous hemopoietic spleen colony-forming units

† E-CFU growth with respect to radiation controls

‡ Confluent colony growth, not quantifiable

Maximum responses were seen in mice injected at 1 hour and at 1 day before irradiation. At those respective times, compared to the 3 ± 1 E-CFU observed in radiation controls, 47 ± 5 and 45 ± 2 E-CFU were observed in the mice treated with 1.5 mg of glucan, and 17 ± 2 and 17 ± 1 E-CFU were observed in the mice treated with 0.4 mg of glucan. The most dramatic hemopoietic responses in postirradiation glucan-treated mice occurred with glucan injected just 1 hour after irradiation. In magnitude, these responses were similar to those observed with glucan injected at 1 hour and at 1 day before irradiation (i.e., 42 ± 4 E-CFU in the 1.5-mg group and 18 ± 3 E-CFU in the 0.4-mg group). However, by injecting glucan consecutively at 1 hour, 1 day, and 2 days after irradiation, an even greater enhancement of E-CFU numbers in postirradiation glucan-treated mice could be produced.

Clearly, these data suggest that glucan may be useful in enhancing the hemopoietic recovery after radiation exposure. Currently, we are assaying the bone marrow and splenic CFU-s, GM-CFC, BFU-e, and CFU-e contents in postirradiation glucan-treated mice.

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RECOVERY PATTERN OF MURINE STROMAL CELLS AND HEMOPOIETIC PRECURSOR CELLS AFTER "EQUIVALENT" DOSES OF NEUTRON OR COBALT-60 IRRADIATION

Principal Investigator: L. M. K. Wathen
Collaborators: T. J. MacVittie and M. L. Patchen
Technical Assistance: J. L. Atkinson and B. Watkins

Preliminary studies indicated that the number of murine stem cells (CFU-s) was depleted to about 1% of the control value following either a neutron dose of 150 rads or a cobalt-60 dose of 400 rads. Thus, we initiated studies to determine if these two doses, which reduce the stem cell population to an equivalent value at 24 hours postirradiation, result in similar patterns of hemopoietic recovery.

Table 1 summarizes the results obtained from total-body irradiation of B6D2F1 mice at a dose of 150 rads of neutrons (150 N) or 400 rads of cobalt-60 (400 C).

Table 1. Recovery of Hemopoietic Stem and Progenitor Cells Following Exposure to Neutron and Cobalt-60 Radiation

Type of Cell	Origin of Cell	Radiation (Rads)	Attained Control Levels by 21 Days Postirradiation
Granulocyte-macrophage colony-forming unit (GM-CFC)	Femur	150 N*	+
		400 C§	+
	Spleen	150 N	+
		400 C	+
Macrophage colony-forming unit (M-CFC)	Femur	150 N	+
		400 C	+
	Spleen	150 N	-
		400 C	+
Erythroid colony-forming unit (CFU-E)	Femur	150 N	+
		400 C	+
	Spleen	150 N	+
		400 C	+
Erythroid burst-forming unit (BFU-E)	Femur	150 N	-
		400 C	+
	Spleen	150 N	+
		400 C	+
Hemopoietic stem cell (CFU-S)	Femur	150 N	-
		400 C	-
	Spleen	150 N	-
		400 C	+
Marrow stromal cell (MSC)	Femur	150 N	-
		400 C	+
	Spleen	150 N	-
		400 C	+

* N, neutron dose

§ C, cobalt-60 dose

(Although only the levels at 21 days postirradiation are outlined, complete measurement of these cells was done at days 1, 3, 7, 9. 14, and 21 after irradiation.)

From these studies we have concluded the following:

1. At these doses, the murine hemopoietic system is less able to repair after neutron damage than after cobalt-60 damage.

2. The hemopoietic stem cells and stromal cells experience the greatest delay in recovery after either cobalt-60 irradiation or neutron irradiation. However, the 150-rad neutron dose caused the most extensive suppression of hemopoietic cells.

3. Stromal cells in the spleen and bone marrow are unable to repopulate after neutron irradiation. This is surprising in light of the relative radioresistance of these cells to X-ray or cobalt-60 irradiation.

* * *

T-CELL EFFECTS ON HEMATOPOIETIC STEM CELLS

Principal Investigator: G. N. Schwartz
Associate Investigators: T. J. MacVittie, M. L. Patchen, L. M. K. Wathen, and S. R. Weinberg
Technical Assistance: J. L. Atkinson, B. Watkins, and D. P. Dodgen

Objectives and Relevancy

The overall objective of this research proposal is to evaluate the role of thymus-derived lymphoid cells (T-cells) in modulating the proliferation and differentiation of hematopoietic stem cells. Survival of an animal after chemical- or radiation-induced aplasia is dependent upon the regeneration of the mature hematopoietic cell compartments by way of stem cell populations. There is evidence of possible T-cell influences on stem cell activity. Thus, to evaluate the various therapeutic strategies after radiation with or without bone marrow transplantation, it is important to know the growth patterns of normal hematopoietic stem cells as related to normal and abnormal T-cells and stem cell interactions.

Summary of Research

Bone marrow and spleen cells enriched with or depleted of thymus-derived cells are being used to study possible T-cell effects on in vitro and in vivo hematopoietic stem cell growth.

Addition of Thymus-Derived Cells

Mixtures of bone marrow and thymus cells from B6D2F1, C3H/HeN, or C57BL/6J mice were assayed for the growth of erythroid colony-forming cells (CFU-E and BFU-E) and granulocyte-macrophage colony-forming cells (GM-CFC). With the addition of thymus cells, only a slight increase was seen in the concentration of GM-CFC. When the marrow-thymus ratio was 1:10 or greater, the number of CFU-E/10^5 marrow cells was not significantly different from that of the control samples (1:0). However, when the marrow-thymus cell ratio was 1:0.02, 1:0.2, or 1:2, the concentration of CFU-E was 125%-200% greater than in the marrow without the addition of thymus cells. An increase in the number of BFU-E/10^5 marrow cells was observed in all cultures in which thymus cells had been added.

Depletion of Thymus-Derived Cells

In vitro complement-mediated cytotoxicity of cells labeled with anti-Thy 1.2 was used to deplete T-cells from the bone marrow. Bone marrow pretreated with monoclonal Thy 1.2 plus complement that was sufficient to kill 50%-89% of cells in the thymus was assayed for the growth of hematopoietic stem cells. No significant difference in the concentration of GM-CFC, CFU-S, CFU-E, or MS-CFU in bone marrow was observed in nontreated cell suspensions, complement-pretreated cell suspensions, or cell suspensions pretreated with anti-Thy 1.2 plus complement.

Preliminary data suggest that there is a significant difference in the recovery of some stem cell populations in lethally irradiated mice that received nontreated bone marrow and in animals that had been transplanted with T-cell-deficient marrow. At days 0, 3, 4, 7, 9, and 14 after bone marrow transplantation, the marrow and spleen cellularities and the numbers of CFU-S, CFU-E, and BFU-E were not significantly different for mice transplanted with nontreated bone marrow, complement-pretreated bone marrow, or bone marrow pretreated with anti-Thy 1.2 plus complement. However, at all days tested, the number of GM-CFC and M-CFC was higher in the recipients of marrow cells that had been pretreated with anti-Thy 1.2 plus complement.

* * *

IN VITRO AND *IN VIVO* ANALYSES OF ISOLATED PROGENITOR CELLS FROM BONE MARROW

Principal Investigator:	J. F. Jemionek
Associate Investigators:	R. L. Monroy, R. L. Chaput, and J. H. Darden
Technical Assistance:	C. L. Feser, S. M. Espay, and C. Hattenberg

Bone marrow cells from canine and primate donors were fractionated by counterflow centrifugation-elutriation (CCE) using a continuous albumin gradient. Three areas of nucleated cell recovery were identified, isolated, and labeled as fractions 1, 2, and 3, respectively (Figure 1) (1). Each fraction was evaluated using in vitro systems (techniques of hematopoietic culture assay) and in vivo systems (bone marrow transplantation and a model for hematopoietic recovery post-irradiation).

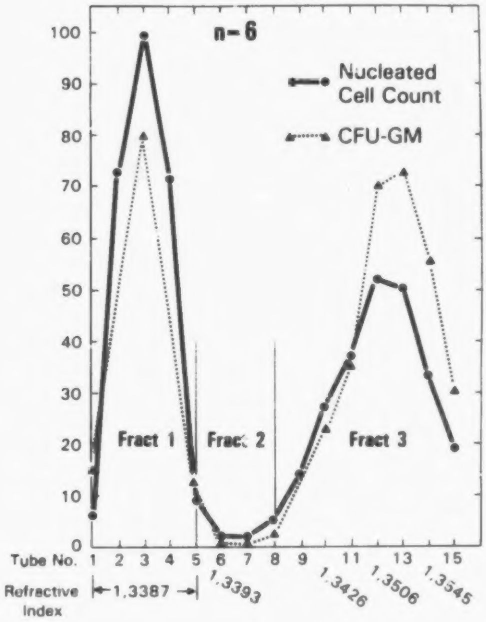

Figure 1. Recovery profile of percent maximal nucleated cell count or GM-CFC activity versus tube number following bone marrow fractionation by CCE. Fraction numbers indicate pooled tube samples used for transfusion of marrow cells back to irradiated animals.

In vitro culture activities [including granulocyte-macrophage colony-forming unit (GM-CFC), marrow stromal cell (MSC), erythroid burst-forming unit (BFU-E), erythroid colony-forming unit (CFU-E), and megakaryocyte colony-forming unit (CFU-MEG)] on fractions 1 and 3 were evaluated. The distribution of culture activities shows a greater percentage of GM-CFC, BFU-E, and CFU-MEG in fraction 1 than in fraction 3. However, fraction 3 had a greater percentage of the CFU-E and MSC activities. GM-CFC, BFU-E, and CFU-MEG are assays of an earlier progenitor cell type (closer to the pluripotent stem cell) than are the CFU-E and MSC assays. This suggests that the pluripotent stem cell and/or earlier progenitor cells are in fraction 1.

Exposure to lethal irradiation (9.0 Gy at 0.1 Gy/minute, cobalt-60) followed by autologous bone marrow transplantation and eventual hematopoietic reconstitution was the model system used to evaluate fractions 1 and 3 *in vivo*. In the canine, transplantation with fraction 1 *survived* the animal, and hematopoietic reconstitution was complete by day 48 postirradiation. This survival and this reconstitution were similar to those observed in animals transplanted with whole bone marrow. By comparison, animals transplanted with fraction 3 showed only limited myeloid repopulation in both the bone marrow and the peripheral blood. The mean survival time of these animals was 24 days (2). In the monkey system, hematopoietic reconstitution and survival have been attained by transplanting fraction 1 postirradiation. The transplantation with fraction 3 is under investigation.

The *in vitro* and *in vivo* data suggest that fraction 1 of *elutriated bone marrow* is enriched in the pluripotent stem cell/earlier progenitor cells, and is capable of hematopoietically reconstituting a lethally irradiated animal. In contrast, fraction 3 contains the more committed progenitor cells, which are capable of only limited hematopoietic reconstitution when transplanted.

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GUINEA PIGS EXPOSED TO 200 RADS GAMMA RADIATION: A COMPARATIVE SEROGENIC AND MORPHOMETRIC EVALUATION

Principal Investigator: G. M. Buchanan

The aims of this study are to (a) determine the effectiveness of ultrastructural morphometric analysis as a measuring tool for evaluating nonspecific cell organelle response to gamma radiation, by comparing the data received by this process to changes in the blood serum and formed elements, (b) establish an index of relative importance that will reveal the potentially best organelles as indicators of cellular response to radiation, (c) evaluate the interrelationship between types of organelles as possible indices of cell response to radiation, and (d) evaluate the possible interrelationships between the organelle changes and the observed peripheral blood changes postirradiation.

The guinea pig (Camm/Hartley) was selected as an animal model because it can supply sufficient blood volume for multiple enzyme analysis, corticotropin, and cortisol radioimmunoassay, and it becomes greatly sensitive to radiation if disease exists. It is important to keep in mind that the changes observed in these animals at 2 Gy are probably due to secondary rather than primary effects of radiation.

Initial $LD_{50(30)}$ data reveal an LD_{50} of 4.5 Gy. After radiation, a crisis phase starts at day 8 and lasts for 8 days. It is during this time period that most deaths occur (Figure 1). Hematologic data revealed extensive alterations. The hematocrit for days 1 through 8 postirradiation was 36.7 ± 0.6; for the controls it was 44.3 ± 1.20. The red blood cell count for the same period was 3.8 ± 0.1 (x $10^9/mm^3$), and for the controls it was 4.4 ± 0.4 (x $10^9/mm^3$). White blood cell count appeared to be biphasic. Up to day 5 postirradiation, it was 1.2 ± 0.1 (x $10^6/mm^3$), and days 6 through 8 yielded a count of 0.6 ± 0.1 (x $10^6/mm^3$). The controls gave a value of 4.8 ± 0.6 (x $10^6/mm^3$). Neutrophils and lymphocytes were not found, generally, in days 1 through 6 postirradiation; they did return to 10%-30% of normal on days 7 and 8. Serum enzyme data yielded significant differences postirradiation in the values for blood urea nitrogen and for the serum levels of glutamic-pyruvic transaminase. The significance of these differences has not been ascertained.

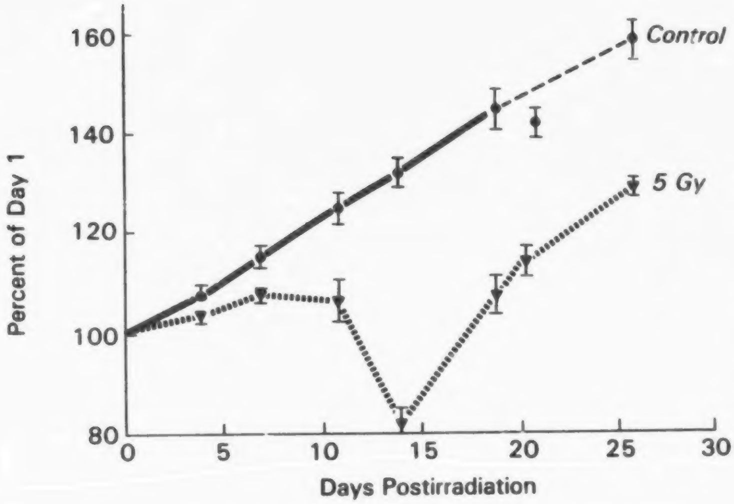

Figure 1. Effect of 5 Gy gamma radiation on guinea pig growth postirradiation

Figures 2 and 3 represent the area-fraction data obtained from the adrenal cortex (zona fasciculata) and the liver, respectively. The area fraction is an estimate of organelle size based on the statistical sampling of a cross-sectional area of a particular organelle. The bars are in terms of the mean ± standard error. Day 0 for both figures represents data obtained from control animals. Both figures show that the area fraction of mitochondria (energy production) is lowest on the second day postirradiation. In the liver, the rough endoplasmic reticulum (protein synthesis) area fraction decreases until the third day, and then begins to recover. This is also the case with the adrenal cortex vacuoles (cholesterol ester storage), which provide the materials from which cortisol is synthesized.

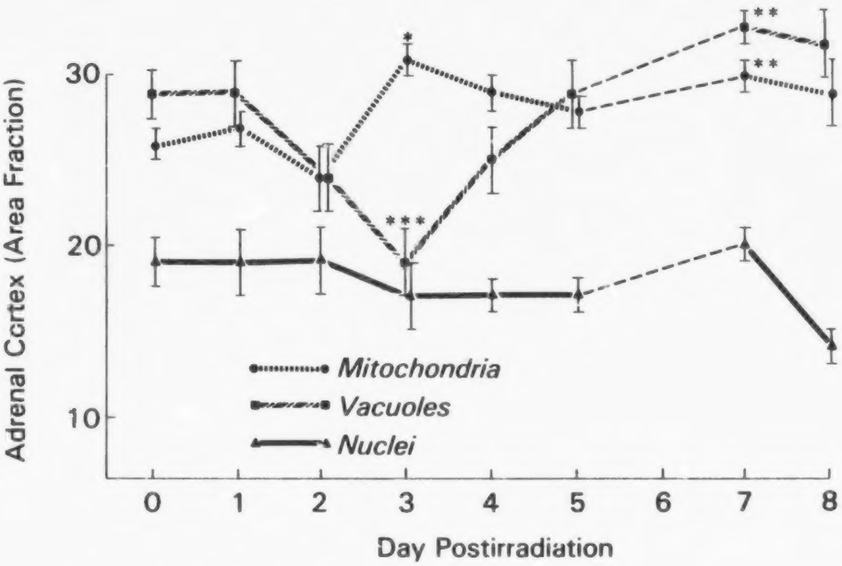

Figure 2. Effect of 2 Gy gamma radiation on area fractions of mitochondria and vacuoles in adrenal cortex of guinea pig. Nuclear area fractions serve as measurement controls.

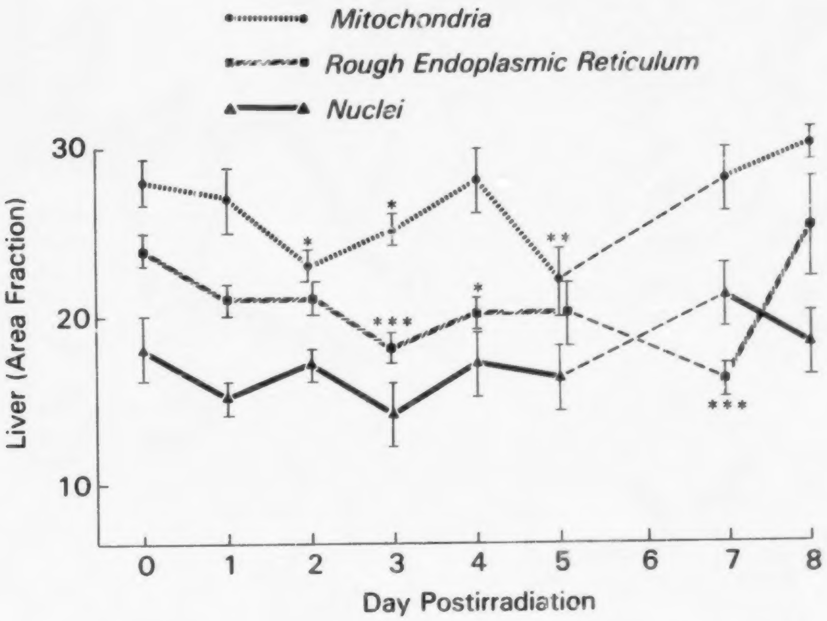

Figure 3. Effect of 2 Gy gamma radiation on area fractions of mitochondria and rough endoplasmic reticulum in liver of guinea pig. Nuclear area fractions serve as measurement controls.

Although the effects of gamma radiation are readily seen, the significance and interrelationships of the observations are not apparent. This work unit is continuing in terms of morphometric analysis of the liver and adrenal cortex as well as further elucidation of serum parameters.

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EFFECT OF COBALT-60 IRRADIATION ON THE MATURATION OF HUMAN MONOCYTE *IN VITRO*

Principal Investigator: V. M. Hartwig
Associate Investigators: W. J. Flor and H. C. Stevenson
Technical Assistance: J. F. Parker and C. L. Feser

The proposed roles of peripheral blood monocytes in antigen presentation (specific and nonspecific), cytotoxicity, and possible hematopoietic regulation are of interest because of the effect of radiation on those roles. This investigation was to identify the morphological changes induced by radiation as the monocyte matures to macrophage in vitro.

Normal human peripheral blood monocytes were obtained by leukapheresis and then purified by counterflow centrifugation-elutriation. Control and irradiated (10.0 Gy, cobalt-60) monocytes were maintained in liquid suspension culture for up to 14 days. Then their maturation from monocyte to macrophage was studied by various microscopy methods: transmission, electron, and scanning. Subjective TEM observations were converted to quantitative terms for statistical analysis by morphometric techniques.

For control monocytes in culture, the total cell volume doubled in size by day 7 as a result of an increase in cytoplasmic volume, with a subsequent decrease in the nucleus-to-cytoplasm ratio. The estimated ratio of outer cell surface to total cell volume remained constant up to day 4 and increased up to 40% thereafter. Irradiation caused an 80% decrease in the nucleus-to-cytoplasm ratio by day 2, but the ratio returned to within experimental error of the nonirradiated control by day 4. The surface-to-volume ratio of irradiated and control cells remained similar throughout the culture. Thus, the macrophage appeared to be relatively resistant to the irradiation under these in vitro conditions, although initial change was observed.

MYELOPROLIFERATIVE CHANGES IN MOUSE HEMATOPOIETIC TISSUES AFTER WOUND TRAUMA

Principal Investigators: G. D. Ledney, D. F. Gruber, D. A. Stewart, and E. D. Exum

Inflammation after surgical or wound trauma is associated with alterations in the number and the functional capacity of mature cells in the peripheral blood. We tested the hypothesis that the lymphomyelopoietic organs reflect proliferative cell changes after the mobilization and utilization of mature cells in the wound-healing processes.

A 4%-body-surface skin wound was made in the anterior dorsal surface of B6CBF1 female mice anesthetized with Metophane. Bone marrow and spleen cells were analyzed at 3, 7, 10, and 14 days after wound trauma for the number of exogenous colony-forming units-spleen (CFU-s), granulocyte-macrophage colony-forming cells (GM-CFC), and macrophage colony-forming cells (M-CFC).

The findings in these experiments were as follows. CFU-s values doubled in the spleen 3 days posttrauma, and were only slightly elevated at the remaining times studied (Figure 1). The first week after wound trauma, the number of marrow CFU-s was reduced to 90% of control CFU-s values. The second week after wound

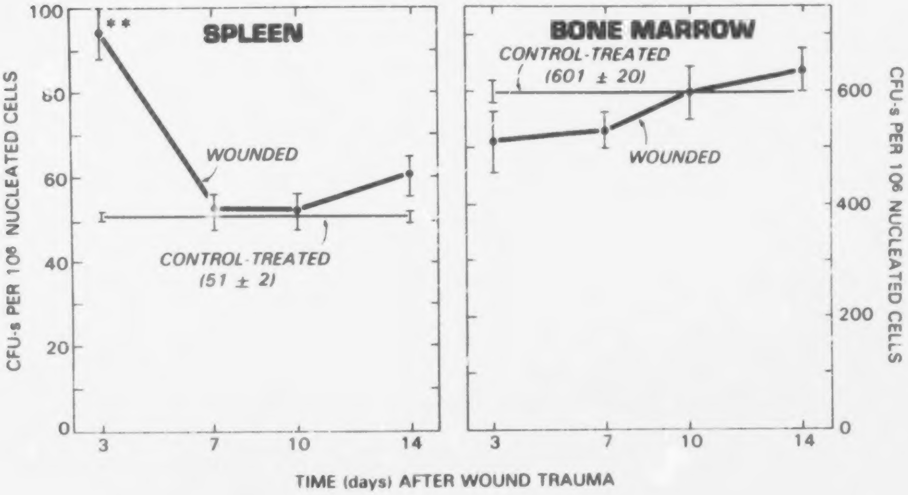

Figure 1. CFU-s concentrations in mouse tissues after skin-wound trauma.
** p = < .01.

trauma, marrow CFU-s quantities were slightly elevated over those of controls. Splenic GM-CFC values increased two- to fivefold in the first week after trauma and were 50% higher than control values during the second week after injury (Figure 2). Marrow GM-CFC numbers were 75% above control values on day 3 posttrauma and were slightly elevated thereafter. The number of splenic M-CFC decreased 25%-50% in the 2-week period after trauma (Figure 3). Marrow M-CFC were elevated 25%-50% throughout the 2-week period after trauma.

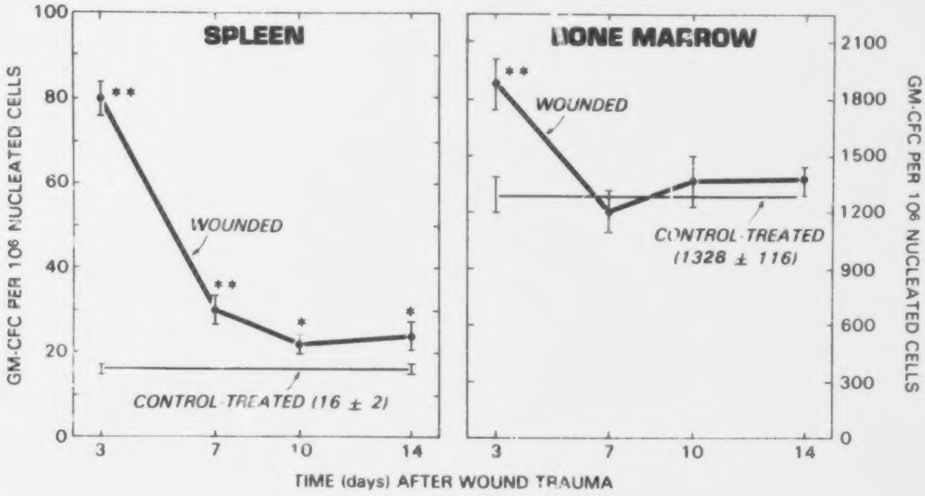

Figure Figure 2. GM-CFC concentrations in mouse tissues after skin-wound trauma.
* p = < .05; ** p = < .01.

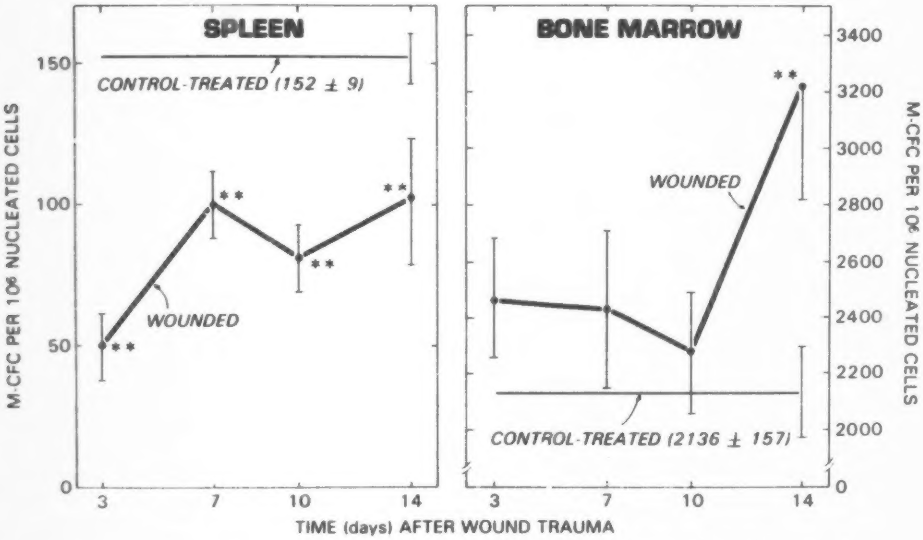

Figure 3. M-CFC concentrations in mouse tissues after skin-wound trauma

In radiation studies (Table 1), endogenous (E) CFU-s were determined in mice given either 700 or 900 rads 24 hours after wound trauma. Wounding before 700 rads resulted in a tenfold increase in splenic E-CFU-s. Mice given 900 rads after wounding had one or two E-CFU-s per spleen, whereas no E-CFU-s were seen in irradiated control animals.

Table 1. Increase in Number of 8-Day Endogenous CFU-s in Male and Female Mice Wounded 24 Hours Before Irradiation

Treatment *†	B6CBF1 (female)	B6CBF1 (male)
Wound 24 hr before 700-750 rads	14.1 ± 1.5‡	5.1 ± 0.6
700-750 rads only	0.5 ± 0.1	1.2 ± 0.3
Wound 24 hr before 900-950 rads	1.7 ± 0.4	0.4 ± 0.2
900-950 rads only	0	0

* Female mice: 700 or 900 rads
Male mice: 750 or 950 rads

† n = 16 in all groups.

‡ ± 1 S.E.

Thus, our data support the hypothesis that myeloproliferative changes occur in the hematopoietic centers after wound trauma. The findings presented may assist in understanding the mechanisms involved in the processes of surgical and wound healing and in the postsurgery scheduling of drug therapies known to be toxic to myeloproliferative cells.

* * *

IN VITRO STUDIES OF LONG-TERM EFFECTS OF RADIATION DAMAGE TO HEMATOPOIETIC GROWTH AND DIFFERENTIATION OF STEM CELLS

Principal Investigator: S. R. Weinberg

The long-term liquid culture technique is being modified for studies of monkey bone marrow. Preliminary data have indicated the importance of the consistency of technical manipulation of bone marrow cells before culture. This culture assay may serve as a means to assess the therapeutic potential of an isolated bone marrow fraction of cells prepared for bone marrow transplantation.

Continued studies with injury to the developing fetus after prenatal exposure to radiation (50-200 rads ^{60}Co to mouse and 90 rads ^{60}Co to dog) have shown significant hemopoietic perturbations in the hemocytopoietic activity of the fetal mouse, fetal dog, neonate mouse, juvenile mouse, and young adult mouse.

Related studies have suggested that the combined trauma of irradiation and surgery to the pregnant dog results in significant changes in the hemograms of peripheral blood and bone marrow.

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PERTURBATIONS IN MURINE HEMOPOIESIS OF FETAL, NEONATAL, AND YOUNG ADULT MICE AFTER PRENATAL LOW-DOSE IRRADIATION WITH COBALT-60

Principal Investigators: S. R. Weinberg and T. J. MacVittie

We investigated the residual hemopoietic effects after irradiating (C57BL/6J X DBA/2J)B6D2F1 mice prenatally (on day 10.5 of gestation) with gamma rays of cobalt-60. The doses were 50 to 300 rads (40 rads per minute), and the ages selected for study ranged from day 14.5 of gestation to 14 weeks of age.

Fetal liver cellularity, morphology, and hemopoietic progenitor cell concentration reflected dramatic injury, beginning with 200 rads. The peripheral blood hemogram, bone marrow cellularity, spleen cellularity, morphology, and hemopoietic progenitor cell concentrations were extended to the 9-day and 15-day neonate and the 14-week young adult. Microplasma clot cultures were used to assess medulla-derived and spleen-derived erythropoietic progenitor cells [erythroid colony-forming unit (CFU-E), erythroid burst-forming unit (BFU-E), and megakarocyte colony-forming cell (Meg-CFC)]. Double-layer soft agar cultures evaluated the concentrations of marrow and spleen granulocyte-macrophage colony-forming cell (GM-CFC).

Pregnant C57BL/6J mice that had received 200 rads showed a greater tendency to abort their litters, and pups of the 150-rad group were generally smaller in size than litters of the same age that had received either a smaller dose of ionizing radiation or no radiation. Observed fluctuations from normal values were greater in the day-15 juvenile than in the day-9 pup, commencing with 50 rads prenatal exposure. This includes the peripheral blood hemogram indices and the marrow and spleen BFU-E's, CFU-E's, and Meg-CFC's. Although neonatal cellularity of spleen reflected greater radiation injury than cellularity of bone marrow, both hemopoietic tissues had significantly reduced numbers of hemopoietic progenitor cells compared to values of the normal neonates. A further reduction in spleen size (compared to normal mice) was observed in the 13-week-old mice that had been irradiated _in utero_ with 150 rads.

Not only did the progenitor cell numbers of irradiated groups of young mice differ from those of nonirradiated mice, but also the values between the male and female mice differed within each group. The effects of prenatal low-dose irradiation are demonstrable in hemopoiesis of the fetal, neonatal, and young adult mouse.

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HEMOPOIETIC PERTURBATIONS IN THE FETAL BEAGLE INDUCED BY LOW-DOSE IONIZING RADIATION AFTER *IN UTERO* EXPOSURE

Principal Investigators: S. R. Weinberg and T. J. MacVittie, *AFRRI*
M. P. McGarry, *Roswell Park Memorial Institute, Buffalo, New York*

Low-dose total-body irradiation to the gravid beagle with cobalt-60 (90 rads, 40 rads/minute) during mid-pregnancy (day 33) was used to perturb fetal hemopoiesis during the third trimester of gestation (days 42-55). Clonogenic culture assays were used to assess the hemopoietic progenitor cells derived from fetal liver, spleen, and bone marrow: the granulocyte-macrophage colony-forming cell (GM-CFC), the erythroid burst-forming unit (BFU-E), and the erythroid colony-forming unit (CFU-E). Peripheral blood was monitored for total nucleated cell counts and differential distribution. Cytospin smears of blood cell-forming tissues were prepared to evaluate recognizable hemopoietic cells.

Peripheral blood counts were 33% lower than normal on day 44, and continued to be lower until day 49 when values became higher than normal. Splenic cellularities of the irradiated pups on day 44 were more than three times that of the nonirradiated, but thereafter they were similar to normal. Differences in hemopoietic activity between irradiated and normal fetuses were observed throughout the third trimester of gestation. Increased marrow CFU-E's and recognizable erythroid cells of irradiated fetuses appeared at the expense of granulocytopoiesis. Although a concomitant decrease was seen in the marrow recognizable proliferative granulocytic cells in the irradiated fetuses, the marrow GM-CFC's were unchanged from normal.

Ten days after irradiation, the spleen contained a marked increase in recognizable nucleated erythroid cells, CFU-E's, and BFU-E's. Similar to irradiated marrow, the irradiated spleen had a decrease in proliferative granulocytic cells, but no fluctuations from normal of GM-CFC's. In contrast, the liver appeared to experience greater radiation injury. For example, on day 44, irradiated liver BFU-E's and CFU-E's were fivefold to tenfold lower than normal, but they recovered to within normal range by day 46. On day 55, the irradiated liver GM-CFC's and granulocytic cells were about 50% lower than normal.

This study contributes information to a highly controversial topic: the effects of low-dose prenatal irradiation.

* * *

POLYETHYLENE GLYCOL PROTEIN COMPLEXES AS RADIOPROTECTORS

Principal Investigator: B. H. Gray
Technical Assistance: R. Stull

Polyethylene glycol 5000 (PEG) was attached to bovine liver catalase, bovine erythrocyte superoxide dismutase (SOD), or bovine serum albumin using cyanuric chloride as a coupling agent (1,2). Compared to unmodified proteins, the PEG-protein complexes have greatly reduced immunogenicity and longer circulating half-lives, when injected into mice (3). The proteins SOD and interferon reportedly have radioprotective activity (4,5). Therefore, the oxygen-detoxifying enzyme complexes PEG-SOD and PEG-catalase were reasonable candidates for trials as radioprotectors.

PEG-protein complexes were injected intravenously into B6CBF1 female and C57BL/6 male mice to determine possible radioprotective effects using cobalt-60 as the radiation source. The results for female B6CBF1 mice given prophylactic injections at 24 hours before irradiation are shown in Figure 1.

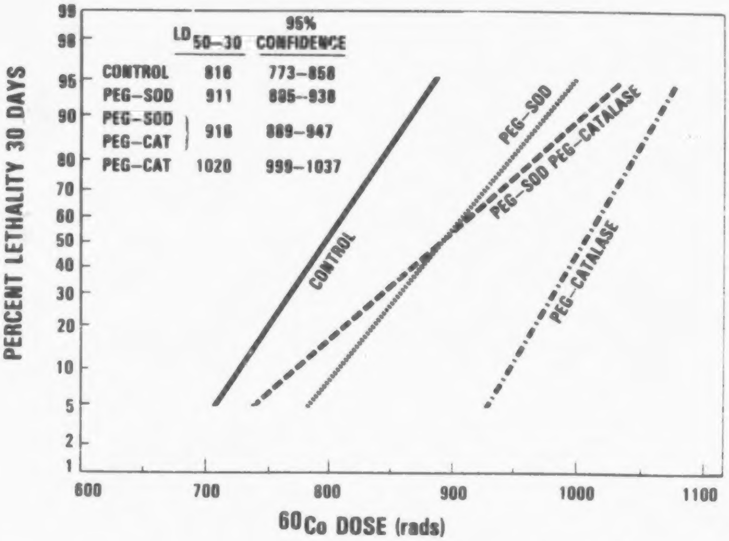

Figure 1. Percent lethality versus cobalt-60 dose for female B6CBF1 mice injected 24 hours before irradiation

The dose reduction factor for 5000 units of PEG-catalase was 1.2. No radioprotective effect was seen for the PEG-protein complexes administered after irradiation. Also, B6CBF1 female mice given prophylactic injections of heat-inactivated PEG-catalase, PEG-albumin, or unmodified catalase had survival rates similar to the phosphate-buffered saline-injected control mice subjected to the same doses of irradiation. No radioprotective effect was seen in C57BL/6 male mice injected with PEG-proteins at either 3 or 24 hours before irradiation.

PEG-catalase and PEG-SOD seem to have a radioprotective effect in B6CBF1 female mice, which depends at least in part on the enzymatic activity of the complexes. However, the radioprotective mechanisms for these oxygen-detoxifying enzyme complexes are no doubt complex, and they depend on the test organism chosen.

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SEROGENIC IMMUNOREGULATORY EFFECTS IN WOUNDED AND IRRADIATED MURINE MODELS

Principal Investigator: D. F. Gruber
Associate Investigator: G. D. Ledney
Technical Assistance: T. A. Davis and E. D. Exum

The experimental study of mechanisms involved in hematopoietic dysfunction(s) requires a basic understanding of the components of the physiological regulation of normal lymphohematopoiesis. The lymphohematopoietic system in most adult mammals is unique by virtue of its capabilities of blastogenic transformation. Lymphocytes are able to dedifferentiate into blast cells. Feedback mechanisms undoubtedly exist, which allow the subject to maintain a "finely regulated" cellular balance. In addition to these "normal day-to-day regulators," others may exist as responses to gross pathological insults.

A feasible military situation may involve persons sustaining various degrees and combinations of wounding and radiation. To investigate the existence of serogenic immunoregulatory substances, we selected a murine model. Within the parameters of the constructed situation, we investigated the response of the cell-mediated immune system to PHA-a T cell mitogen and LPS-a B cell mitogen. Measures of blastogenic transformation gave us functional information on lymphocyte responses as determined by the incorporation of ^{3}H-TdR into DNA. Mice were age-matched (12 weeks old) female (C57BL/6 X CBA)F1 Cum BR. Circular skin wounds of 2-2.5 cm^2 were induced into the anterior dorsal skin fold using an alcohol-sterilized steel punch. Selected radiation levels were 600-900 total rads at 50-rad increments at a standard dose rate of 40 rads per minute. Only cobalt-60 gamma irradiation was investigated.

Animals were bled and their serums were pooled at days 1-7, 10, 14, 21, 28, 35, and 42 posttreatment. The serums of wounded animals were contrasted to control levels. The lymphocyte blastogenic transformation assay, compared to controls, demonstrated significant immunosuppression through 21 days, whereupon gradual increases toward normal were noted. Serum factors were evaluated in (C57BL/6 X CBA) female mice receiving 600-850 rads of cobalt-60 radiation and surviving for 30 days. Animals in the 600-850-rad range who survived for 30 days were exsanguinated. Pooled portions of this blood were examined for immunosuppression. It was determined that even after 30 days, irradiated surviving animals have sufficient serogenic immunosuppression to inhibit responses to both lipopolysaccharide and phytohemagglutinin. Lipopolysaccharide was suppressed by an average of 64%, and phytohemagglutinin was suppressed by 25%.

RESPONSE OF C-REACTIVE PROTEIN IN MICE IRRADIATED WITH COBALT-60 AND FAST NEUTRONS

Principal Investigator: H. M. Gelston, Jr.
Associate Investigator: G. D. Ledney

Tissue damage in mammals results in an acute inflammatory response. One facet of this response is the liver's increased synthesis of certain serum proteins. One of these proteins, C-reactive protein (CR-P), has been shown to be an excellent indicator of tissue damage and sepsis in humans. High levels of radiation exposure will result in tissue damage. Our efforts have been directed toward characterizing the C-RP response of laboratory animals to (a) various levels of cobalt-60 irradiation, (b) fast neutron irradiation, and (c) combined injury.

A protocol for determining the serum C-RP levels in mice and dogs was developed. This procedure uses endpoint laser nephelometry and the cross-reaction of antihuman C-RP with murine C-RP and canine C-RP. The majority of our work dealt with the murine response. These preliminary data will be presented here.

B6CBF1 male mice were lethally irradiated with either 1200 rads of cobalt-60 or 600 rads of fast neutrons (Figure 1). Significantly elevated levels of C-RP were detected 24 hours later. However, the C-RP levels of mice irradiated with 700 rads of cobalt-60 or 100 rads of fast neutrons remained basically within normal limits (Figure 1). This indicates that higher doses of either type of radiation produced tissue damage. That was significant enough to elicit an acute-phase response. In addition, the lethally irradiated mice showed elevated C-RP levels before death. The mice irradiated with 600 rads of fast neutrons showed increased levels of C-RP throughout the 4-day monitoring period. Mice that had been irradiated with 1200 rads of cobalt-60 had C-RP levels that returned to normal after the initial response. However, C-RP levels were markedly elevated from day 6 postirradiation until death at day 12. These data suggest that sepsis or endotoxin shock, both potent stimulators of C-RP synthesis, may play a role in the events that lead to death in lethally irradiated mice.

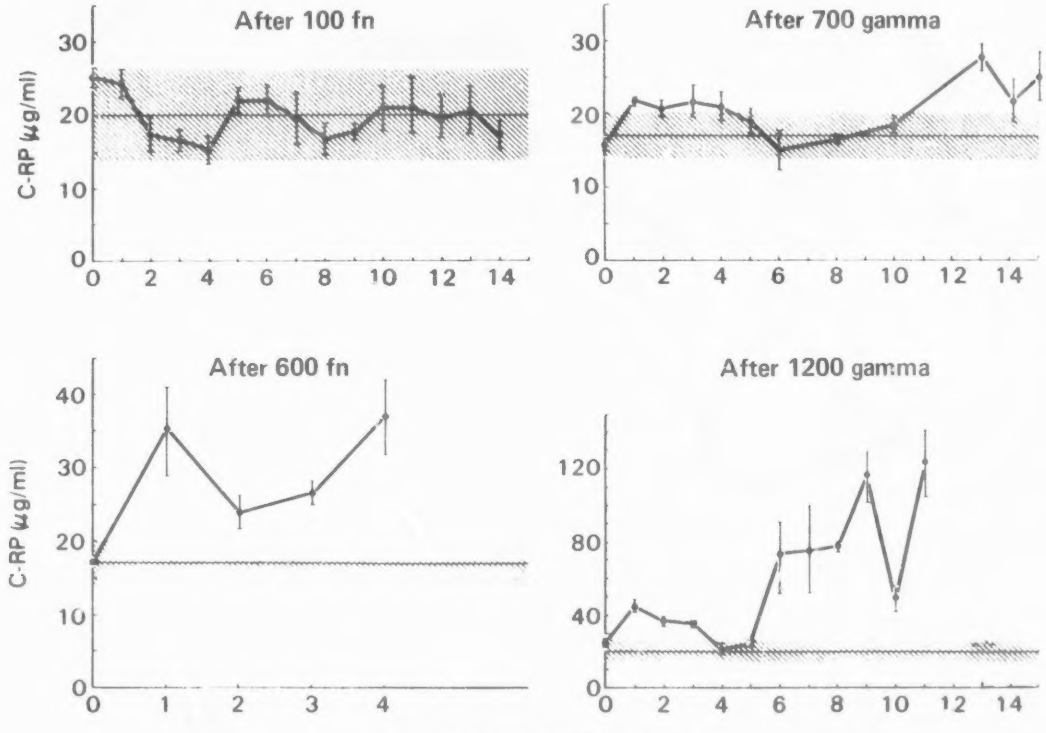

Figure 1. Serum C-reactive protein levels determined for B6CBF1 male mice at 24-hour intervals after irradiation. Assays were carried out for 14 or 15 days or until death (1200 rads cobalt-60 and 600 rads fast neutrons). C-RP levels in μg/ml are shown only for comparison purposes. Since our assay uses a polymer enhancer (PEG), the values obtained are probably elevated. However, the patterns displayed indicate the acute-phase response to inflammation.

The data presented suggest that C-reactive protein may be an indicator of radiation exposure and may be an effective parameter that can be used to monitor the efficacy of postirradiation therapeutic measures.

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POSTIRRADIATION CHANGES IN GRANULATED LEUKOCYTE NONHISTONE CHROMOSOMAL PROTEINS

Principal Investigator: M. J. Taylor

Nonhistone chromosal proteins (NHCP's) have been implicated in the regulation of cell proliferation and in the function of cells of many developing organs *in vivo*, including cartilage, liver, brain, and hormone-responsive tissues as well as hematopoietic cells *in vitro*. It is precisely those cells undergoing differentiation and division that are most susceptible to damage by radiation. It may be possible to use white cell-specific NHCP's to initiate or control the differentiation and hence the function of these cells.

During the past year, three closely related white cells have been purified for the purpose of isolating the cell-specific NHCP's. Neutrophils have been routinely recovered at the 95%-98%-purity level in the laboratory, using a discontinuous percoll gradient. Macrophages can now be prepared routinely with 99% purity using elutriation, followed by a release of adherent cells in 0.02% EDTA at 37°C. Previous procedures using either adherence or elutriation alone yielded 90% purification. Because a 10% cell contamination can yield a protein of considerably greater contamination, greater cell purity was essential.

An eosinophil purification of 95% has been achieved with a combination of elutriation and discontinuous percoll gradient containing catalase, thus reducing the cell breakage due to peroxide release from damaged cells. Previous preparations of rodent eosinophils in the literature included 88% purification but with 40% recovery due to loss of adherent eosinophils, or 95% purification but with 1200 rads of irradiation the day before cell harvest. Neither procedure is suitable for isolating acidic protein because it is not desirable to (a) selectively exclude a functional population of cells, or (b) use irradiated cells in protocols in which cell-specific macromolecules are to be examined in irradiated animals. Nuclei have been purified for the neutrophil and macrophage. Once eosinophil nuclei are obtained in sufficient quantities and purities, the NHCP comparisons will be made to determine if specific nuclear proteins are damaged by radiation.

USE OF THE BrdUrd/313-NM LIGHT TECHNIQUE TO ANALYZE DEVELOPMENT OF BONE MARROW

Principal Investigators: M. P. Hagan and T. J. MacVittie

We have used the BrdUrd/313 nm light technique to demonstrate and quantify the cycling of bone marrow-derived spleen colony-forming units (1). During the past year, we have undertaken the analysis of the bone marrow-derived colony-forming unit (GM-CFCc).

Long-term, low-level BrdUrd infusion identifies two subpopulations of GM-CFCc with quite dissimilar sensitivities to 313 nm light. The responses of these two GM-CFCc subpopulations to hydroxyurea indicate that both are rapidly proliferating at the time of the assay. However, the absolute ultraviolet-light sensitivity of the S-phase components and the effects of increasing the BrdUrd concentration indicate that the two GM-CFCc subpopulations passed through the previous cell cycle at widely disparate rates. Further, these GM-CFCc, originating from a parental cell with a slow turnover, are associated with a lower buoyant density than the GM-CFCc that have been in rapid cycle for at least two generations. These results indicate that the resistance to 313 nm light irradiation, shown by S-phase cells in the first cycle of the BrdUrd labeling, may provide evidence of the proliferative history of the cell being assayed.

Shown in Figure 1 are the cell survival curves of GM-CFCc assayed after 5 or 8 days of BrdUrd infusion. Panel A shows the effect of treatment with hydroxyurea (●) to eliminate the S-phase population of the otherwise normal GM-CFCc (○). By subtracting the survival values of the hydroxyurea-treated cells from the survival of controls, the "difference curves," which are the survival curves of the S-phase components (shown in panel B), depict a population with the characteristic shoulder region of cells in the first cycle of BrdUrd labeling.

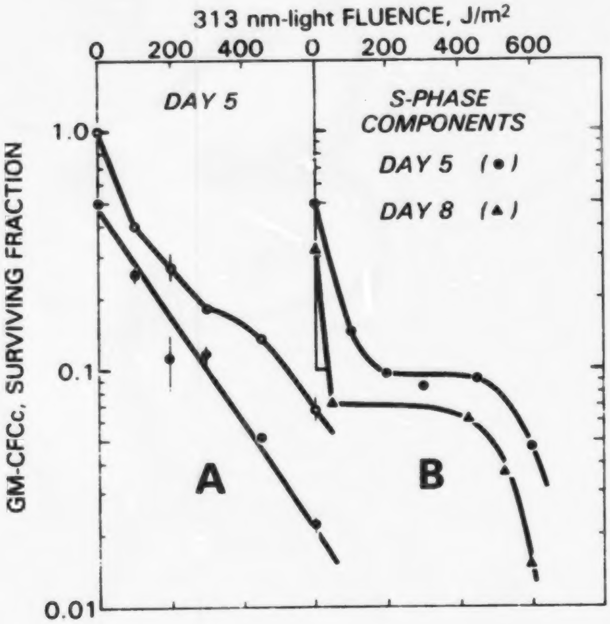

Figure 1. Combined hydroxyurea and 313 nm-light response for cells irradiated after 5 days of BrdUrd infusion. Infusion was 2 mg/kg-hour from subcutaneously implanted minipumps. A: Cell suspensions were treated for 2 hours with medium containing hydroxyurea (●) or medium alone (○) before exposure to 313 nm light. B: S-phase survival was determined by subtracting survival values shown in panel A (●). Same process was also used to obtain data shown for 8 days of BrdUrd infusion (▲) (survival curves not shown).

Thus, these data showed for the first time that GM-CFCc enter the cell cycle from a noncycling pool. This demonstration is an important clue to the organization of development of bone marrow.

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STUDY OF MOLECULAR EVENTS ASSOCIATED WITH DNA REPAIR OF BrdUrd/313-NM LIGHT LESION(S)

Principal Investigators: M. P. Hagan and H. M. Jacocks

It has been previously shown that cysteamine, when present at the time of irradiation with 313 nm light, can reduce the lethality of the ultraviolet exposure for BrdUrd-labeled mammalian cells (1). This year we investigated the cell-cycle response of this protective effect. These results (shown in Figure 1) indicate that the administration of cysteamine during periods of efficient deoxyribonucleic acid (DNA) repair actually sensitizes BrdUrd-labeled cells to ultraviolet light. Several explanations are possible for this effect, and the most plausible is the chemical cross-linking of cysteamine to DNA. This process is known to occur, but previously has been thought to be relatively inocuous. This problem has not been addressed using synchronous cell cultures. The sensitivity present in the cell-cycle analysis may permit the evaluation of this form of DNA damage. Since this damage was potentiated through the presence of caffeine, we examined the effects of caffeine on the kinetics of the removal of alkali-labile lesions and uracils after treatment with BrdUrd/313 nm light. Caffeine appeared to have no direct effect on the repair kinetics of these lesions (2).

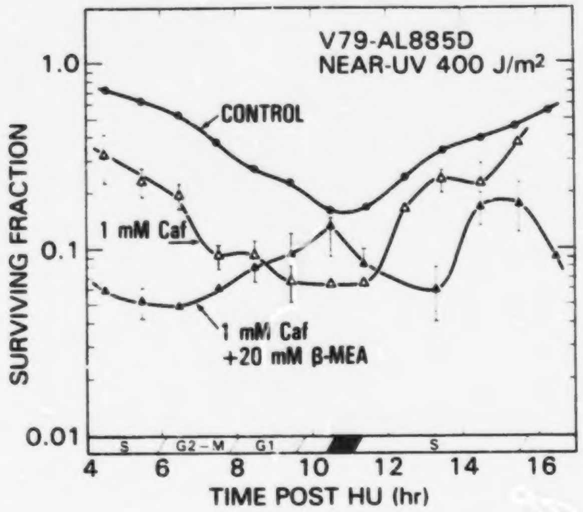

Figure 1. Age response after BrdUrd/near-UV light treatment. V79-AL885D cells were irradiated with 400 J/m^2 of near-UV light at time of cell cycle shown. Cysteamine (B-MEA) in presence of 1 mM caffeine (Caf) enhanced cell killing at those positions of cell cycles associated with normally efficient DNA repair.

To further investigate this and other similar reactions, we have established a laboratory for high-performance liquid chromatography. This laboratory will focus on the isolation of DNA addition products. It is the specific aim of this laboratory to isolate and characterize deoxyribonucleosides from samples not exceeding 1 x 10^7 cells. This limit on the sample size will afford the use of synchronized cells. Further, the resolution of this technique is designed to be one altered based per thousand bases. Enzymatic isolation techniques have been the focus thus far. Figure 2 is a normal chromatogram for DNA extracted from 1 x 10^7 Chinese hamster cells.

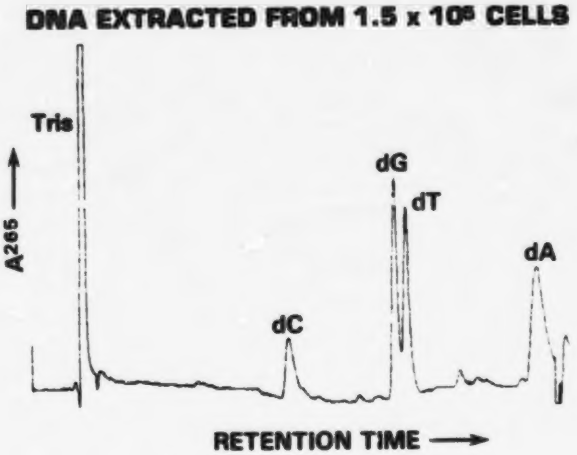

Figure 2. Enzymatically hydrolyzed DNA. V79-A03 cells (1.0×10^7 cells) were lysed by detergent action, the protein and RNA were enzymatically hydrolyzed, and the DNA was isolated by hydroxyapatite chromatography. Isolated DNA was enzymatically reduced to nucleosides and chromatographed by reverse-phase chromatography on a C-18 column.

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PHYSIOLOGY DEPARTMENT

The responsibility of the Physiology Department is to design and execute research toward understanding the effect of radiation or related insults on the physiological function of biological systems. The department uses an integrated approach in pursuing these studies, which ranges from single cells in tissue culture isolation to awake, performing animals.

The Department is divided into three major Divisions, with research interests as follows:

Studies in the General Physiology Division concern the investigation of effects of mid-level and high-level doses of radiation (500-10,000 rads) on the cardiovascular and gastrointestinal systems. The acute effects of exposure to this range of supralethal radiation is of particular interest to military commanders as they plan for operations in a nuclear warfare environment. The performance capabilities and limitations of combat troops hold the key to either success or failure of the mission and ultimately to survival. Radiation-induced hypotension has been implicated as the cause of early transient incapacitation (ETI), which is found after exposure to mid-level and high-level radiation. One study of radiation-induced hypotension includes the measurements of blood volume, cardiac output, arterial pressure, central venous pressure, and mean circulatory filling pressure. Another study addresses the same problem by correlating changes in systemic blood pressure, intestinal blood flow, and plasma histamine levels.

Radiation-induced acute histopathological and ultrastructural changes in the intestinal mucosa are being correlated with changes in blood flow and pressure, thereby addressing at once the two problems of radiation-induced cardiovascular collapse and gastrointestinal dysfunction. To further elucidate the etiology of ETI, an attempt is being made to develop and characterize an animal model for study of the acute effects of focally directed radiation to the cerebrovascular system.

Research within the Neurophysiology Division is directed toward tasks of the highest priority within the AFRRI 5-Year Plan: study of the mechanisms of performance decrement and incapacitation and the combined effects on combat performance. The breadth of research ranges from the single nervous-system cell to the awake, performing monkey. Excitable cells are grown in tissue culture where they can be studied in great detail. Studies are being conducted to determine if radiation alters the ion channels responsible for the neurophysiological behavior of single cells. Cultured neurons with nicotinic and muscarinic acetylcholine receptors are being used to study the mechanisms of action of neurotoxic chemical warfare agents and how radiation may interact with these chemical effects.

From single cells, another order of complexity can be achieved using isolated brain slices. Studies similar to those above are being done with these preparations. Furthermore, various substances that may be released from nonneuronal cells, such as mast cells, are being examined for effects on neuronal excitability. Finally, the next level of organization being used is the awake mammal. Nonhuman primates are taught specific tasks, and the effects of radiation, focally applied to the spinal cord, are studied.

The research efforts of the Radiation Biophysics Division are multifaceted, and are focused on understanding the effects of radiation at the cellular level. One of its research goals (directed toward the task forces designated to study the effects of radiation-induced hematopoietic dysfunction as well as medical therapy of combined effects) is devoted to elucidating the effects of radiation on the macrophage. These cells, although relatively radioresistant in terms of survival, are long-lived, and play a pivotal role in immune surveillance and host defense. Techniques of cell culture and biophysics are being used in conjunction with functional assays to describe the effects of radiation on the macrophage.

A second area of research within the Division is the study of radiation-induced gastrointestinal dysfunction. Sublethal doses of ionizing radiation produce incapacitation through vomiting, diarrhea, and nausea, whereas supralethal doses result in denudation of the intestinal mucosal lining and also bacterial toxemia. Electrical and transport measurements are being used to study the cellular effects of radiation in intestinal epithelium.

The third and last major area of research of this Division is the study of the mechanisms of ionic recovery following radiation. This area of research is a part of the AFRRI task force directed toward understanding the mechanisms of performance decrement and incapacitation.

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LEARNED CHANGE IN SPINAL STRETCH REFLEX: EFFECTS ON MOVEMENT AND ANTAGONIST MUSCLE BEHAVIOR

Principal Investigator: D. J. Braitman, *AFRRI*
Collaborators: V. A. Kieffer, *AFRRI*
J. R. Wolpaw, *New York State Department of Health, Albany, New York*

We have been investigating the effects of ionizing radiation on the electrical activity of the central nervous system. We found that after localized irradiation to the cervical spinal cord, an increase occurs in the spinal stretch reflex (SSR) from the biceps muscle, as measured by the electromyogram (EMG). In order to relate these changes in SSR to motor behavior, we examined concurrent changes in movement and antagonist muscle activity in animals that had been trained to change SSR amplitude without changing initial muscle length or background EMG.

Five monkeys learned to keep elbow angle at 90° ($\pm 1.5^{\circ}$) against steady extension force. If this angle was held for a randomly selected period of 1 to 2 seconds, and if the average absolute value of biceps EMG (from chronic intramuscular electrodes) for the final 0.2 second was 1.0-1.5 times a set value, then a brief pulse of force extended the elbow 3°-4°. SSR amplitude was measured as the average absolute value of biceps EMG 12.5-21.5 mseconds after pulse onset, minus background EMG amplitude. Under the control condition, reward occurred at 100 mseconds. Reward occurred only if SSR amplitude was more (SSR↑) or less (SSR↓) than a criterion value. Control condition data were obtained for 10-50 days. SSR amplitude was stable over this period. Then the animal was exposed to the SSR↑ and SSR↓ conditions in succession for prolonged periods.

Animals appropriately increased or decreased the SSR amplitude as required over 10-20 days (1). Arm movement for the first 40 mseconds after the brief pulse of force did not change when changes were made in the SSR amplitude. After 40 mseconds, movement was inversely related to amplitude of the biceps SSR (Figure 1).

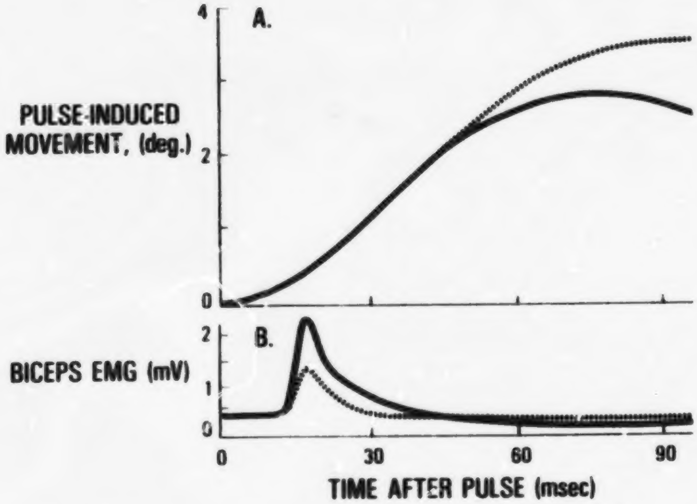

Figure 1. Pulse-induced movement (A) and biceps EMG amplitude (B) from one animal. Solid line is a day's data (>3000 trials) under the SSR↑ condition; dashed line, a day's data under the SSR↓ condition. Background (prepulse) EMG and first 40 msec of movement are the same for the 2 days.

It appears that SSR is responsible for deceleration of the arm following a pull to or stretch of the arm. Thus a larger SSR (Figure 1B) results in a quicker deceleration of the arm (Figure 1A) (i.e., less pulse-induced movement), whereas a smaller SSR (Figure 1B) results in slower deceleration and greater pulse-induced movement (Figure 1A). The 20- to 25-msecond delay between SSR and its apparent effect on arm movement was presumably the time necessary for muscle contraction after excitation.

Triceps EMG was minimal under all three conditions. Thus the antagonist muscle does not appear to play a major role in the change of SSR amplitude or in the accompanying post-40-msecond change in movement.

Based on these results, it appears that the increase in SSR amplitude after irradiation of spinal cord may significantly affect motor control and dexterity. Several monkeys are being trained, and will be irradiated for comparison with these data. This experimental model will allow us to determine the effects of ionizing radiation on motor synaptic functions, in producing the degradation of combat performance.

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ACTIONS OF METHYLTETRAHYDROFOLATE AND KAINIC ACID ON ELECTROPHYSIOLOGICAL ACTIVITY IN BRAIN SLICE OF THE OLFACTORY CORTEX

Principal Investigator: D. J. Braitman
Collaborator: S. L. (Rubinstein) Brown
Technical Assistance: E. Sølberg

We have been investigating the neuropharmacological substrates of changes in electrical activity in the central nervous system following ionizing radiation. In this effort, we have extended our experiments on the actions of excitotoxic amino acids on isolated slices of brain tissue from the olfactory cortex. In a prior study (1), we compared the effects of methyltetrahydrofolate (MTHF), a naturally occurring amino acid derivative, to the effects of kainic acid. Kainic acid is an exogenous amino acid that has excitatory actions on the olfactory cortex similar to those of ionizing radiation (2). MTHF was reported to be a specific competitor for kainic acid-binding sites in rat cerebellar membranes. Since MTHF does not appear to have the same excitatory action that kainic acid has in olfactory cortex (1), we examined the ability of MTHF to block kainic-induced excitation.

Thin slices (300-400 μm thick) of rat olfactory cortex were cut and incubated at 35°C in oxygenated normal Ringer's solution. Each slice was transferred to a recording chamber that was constantly perfused with Ringer's solution. The lateral olfactory tract (LOT) was stimulated with double shocks of 20-50 volts for 50 μseconds via a tungsten bipolar electrode placed across the tract. Paired shocks were separated by 60 mseconds and administered every 4 seconds. Shock intensities were adjusted to produce field potentials of maximum amplitude. Glass micropipettes with approximately 50-µm cut tips were filled with normal Ringer's solution, and used to record field potentials from the pial surface of the slice. After recording stable control field potentials for several minutes, the perfusate was changed to a Ringer's solution containing kainic acid, MTHF, or kainic acid plus MTHF. Concentrations of these agents ranged from 10^{-8} M to 10^{-3} M; perfusion times ranged from 5 to 30 minutes. This was followed by a wash in normal Ringer's for a period of time equal to or greater than that of the drug treatment.

As previously reported (1), kainic acid showed dose-dependent excitatory effects on LOT-stimulated field potentials at concentrations greater than or equal to 5 x 10^{-7} M (Figure 1C), whereas MTHF had little or no effect even at 10^{-4} M (Figure 1B). Since biochemical assays indicated that MTHF is a potent competitor for kainic acid-binding sites in rat cerebellum (3) but does not appear to be an agonist in rat olfactory cortex (1), we tested the possibility that MTHF is an antagonist (blocking agent) at these kainic acid receptors. MTHF (10^{-4} M) and kainic acid (5 x 10^{-6} M) were individually and then simultaneously bath-applied to the slice during LOT stimulation.

Kainic acid alone decreased the amplitude of the excitatory postsynaptic potential and increased the amplitude of the population spike, whereas MTHF alone had no effect. In combination, MTHF neither diminished nor slowed the effects of kainic acid on the excitatory postsynaptic potential or the population spike (Figure 1). Thus, MTHF does not appear to be an antagonist at the kainic acid receptor in olfactory cortex.

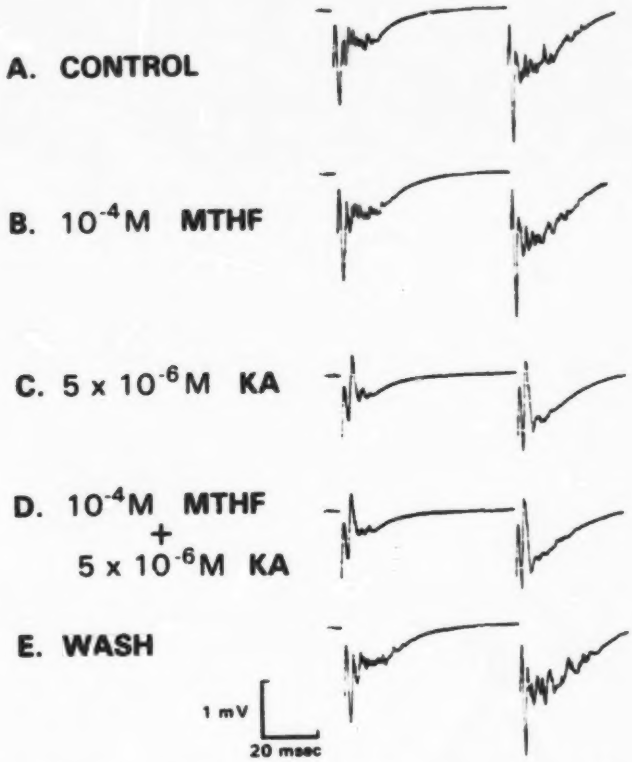

Figure 1. Lack of agonist and antagonist activity of MTHF in rat olfactory cortex slices. A: Control: Cortical field potentials evoked by double shock stimulation to lateral olfactory tract (LOT). B: Effect of 10-min superfusion with 10^{-4} M MTHF. C: Effect of treatment for 8 min with 5×10^{-6} M kainic acid (KA). D: Effects of combined treatment for 7 min and 30 sec with both 10^{-4} M MTHF and 5×10^{-6} M KA. E: Wash (31 min). MTHF was prepared in oxygenated Ringer's just before use and was protected from light during experiment.

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ELECTROPHYSIOLOGICAL AND PHARMACOLOGICAL CHARACTERISTICS OF THE SEROTONIN RESPONSE ON A VERTEBRATE NEURONAL SOMATIC CELL HYBRID

Principal Investigators: J. E. Freschi, *AFRRI*
W. G. Shain, *New York State Department of Health, Albany, New York*

We studied the effects of 5-hydroxytryptamine (5-HT, serotonin) on the vertebrate neuronal somatic cell hybrid TCX11. We compared the electrophysiological and pharmacological properties of the 5-HT response to those of the dopamine (DA) response.

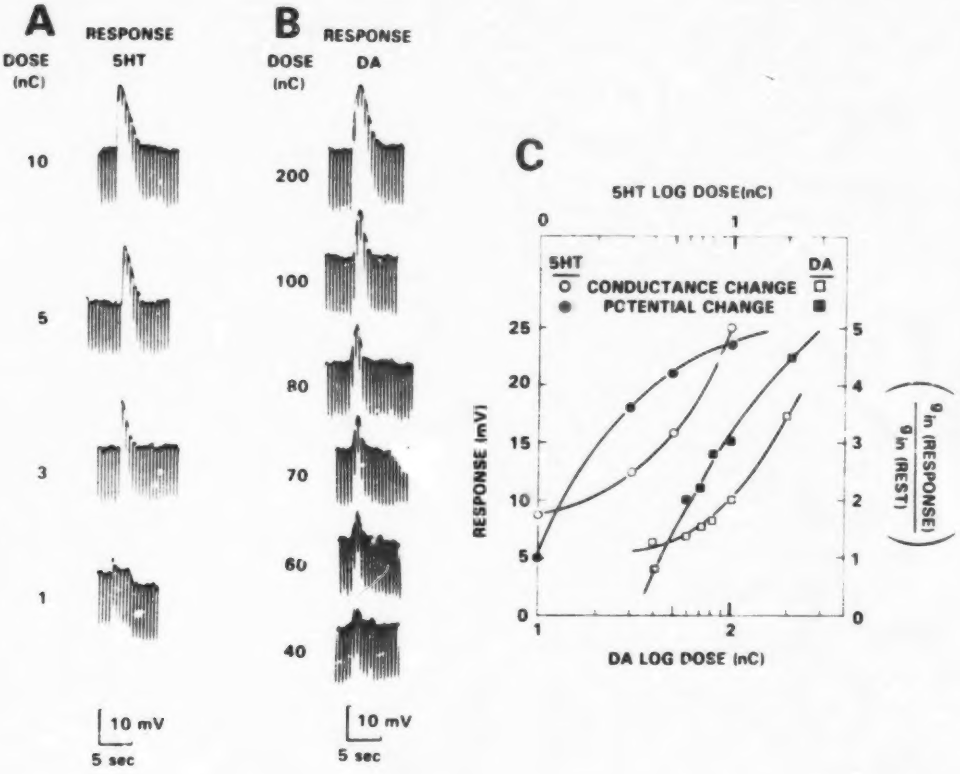

Figure 1. Dose-response relationships for 5-HT and DA on a TCX11 cell. A: Voltage and input resistance changes evoked by 5-HT. Amount of charge iontophoresed from 5-HT pipette is indicated to left of each trace. Resistance was monitored by repetitively passing hyperpolarizing constant current pulses across membrane. B: Voltage and input resistance changes evoked by DA. Traces were generated as in A. C: Graphic representation of A and B. Note different scales for DA and 5-HT abscissas. Lines were drawn by eye.

The cells developed delayed rectification and the ability to generate action potentials. Both 5-HT and dopamine caused graded depolarizations associated with increases in membrane conductance. However, the cells were 10 to 100 times more sensitive to 5-HT than to dopamine (see Figure 1). Responses to the amines did desensitize during sequential or continuous application, and each neurotransmitter could desensitize the cell to a subsequent dose of the other neurotransmitter (cross-desensitization). Reversal potentials for both amines ranged from 0 to 15 mV from cell to cell, but on any given cell, the reversal potentials for 5-HT and dopamine were equal. We applied a variety of drugs, including classical dopamine and 5-HT antagonists, and found no drug that blocked the response to one amine without a similar inhibition of the response to the other amine (Figure 2).

We conclude that, on the hybrid TCX11, there is a receptor that mediates a depolarizing response with a conductance increase after interaction with 5-HT and dopamine. Our data suggest that the receptor is best classified as a serotonin receptor.

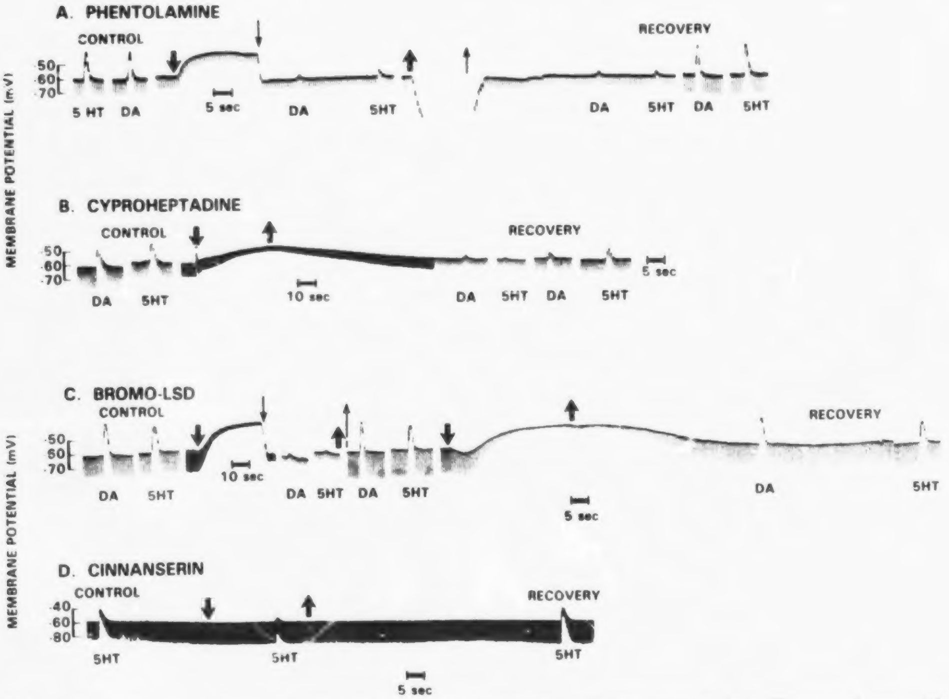

Figure 2. Effects of drugs applied by a perfusion pipette. Experiments A to D were done with different cells. In each experiment, large arrows indicate application and withdrawal of test drug; small arrows show passage of steady inward current to hold cell at about -60 mV. Of the four drugs tested in this way, only cinnanserin blocked 5-HT (and DA) response without having direct effect on membrane conductance (D).

* * *

EFFECT OF GAMMA IRRADIATION ON TRANSMITTER RELEASE FROM FROG NEUROMUSCULAR JUNCTION

Principal Investigator: D. R. Livengood

At relatively low to intermediate doses of radiation, including levels that are used for therapeutic treatment of malignancies in humans (50-150 rads), pronounced fatigue follows the radiation exposure. This fatigue may last for an extended period of time, and can be very debilitating. The mechanism(s) underlying that fatigue is unknown. It is not clear if this perception is psychological, mediated by the central nervous system, or a malfunction of the neuromuscular junction. A failure of neuromuscular transmission at either the pre- or postsynaptic component of the neuromuscular junctions would lead to a marked sensation of fatigue as in the myasthemic syndrome or in myasthemia gravis.

To examine the role of the neuromuscular junction as the site of radiation-induced fatigue, the neuromuscular junction of the frog was used as an experimental model. This preparation is preferable to a mammalian preparation because it is less prone to physical and thermal damage as a result of the manual manipulations before radiation exposure.

Matched pairs of Sartorius muscles were dissected from the frog *Rana p. pipiens*. Both muscles were taken to a cobalt source in a tube filled with normal frog saline (1). One muscle of the pair was exposed to 10,000 rads of gamma radiation at 2,000 rads/minute for 5 minutes. The other muscle was treated in the same way except it was not exposed to the cobalt source. Both muscles of the pair were then pinned in a recording chamber, and standard electrophysiological techniques were used to record from the neuromuscular junction (2). Tetrodotoxin was added to the frog saline so that any change in the normal excitable sodium channels would not affect the transmitter-releasing mechanism of the neuromuscular junction. Neostigmine was added to partially block the postsynaptic acetylcholine esterase as it degrades the acetylcholine released from the junction; this made the spontaneously occurring miniature endplate potentials (MEPP's) larger and easier to record.

MEPP's were recorded on a strip chart recorder. The frequency of MEPP release is a measure of the presynaptic release mechanism, and is a function of the concentration of internal calcium. MEPP amplitude is a function of the integrity of the postsynaptic receptor, the degrading mechanism, and the content of transmitter contained in the synaptic vesicles. MEPP frequency was determined for 13 neuromuscular junctions from control muscles and 13 neuromuscular junctions from irradiated muscles. Table 1 shows the average MEPP frequency for both groups of muscles. The irradiated junctions showed a 57% decrease in the rate of release of MEPP. Qualitatively, no change in the amplitudes of the MEPP's was seen, although this was not studied in detail.

Table 1. Average MEPP Frequency

Control	Irradiated
0.84* ± 0.12†	0.36* ± 0.067†

* Average frequency in MEPP's/second

† Standard error of the mean

These results indicate that some mechanisms in the normal release process are altered by radiation. This may involve the mechanism controlling the concentration of internal calcium, or it may be due to a direct effect on the release sites of the ending.

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EFFECT OF RADIATION ON MACROPHAGE FUNCTION

Principal Investigator: E. K. Gallin
Collaborators: J. H. Darden and S. W. Green

The phagocytic ability of thioglycollate-induced peritoneal macrophages (MØ) from mice that had been irradiated with doses of 5 to 20 grays of cobalt-60 was evaluated at varying times postirradiation.

Bovine red blood cells (E) opsonized with antibovine IgG were added to cultures, and the percentage of MØ containing E(IgG) and the phagocytic index (the percentage of phagocytic cells times the average number of E-ingested per MØ) were determined visually. These radiation doses produced decreases in the percentage of phagocytic cells, and reduced the phagocytic index of the cultures at 6 to 10 days postirradiation (Figure 1). On day 3 postirradiation, only a small decrease in the phagocytic index of irradiated cultures was noted (1).

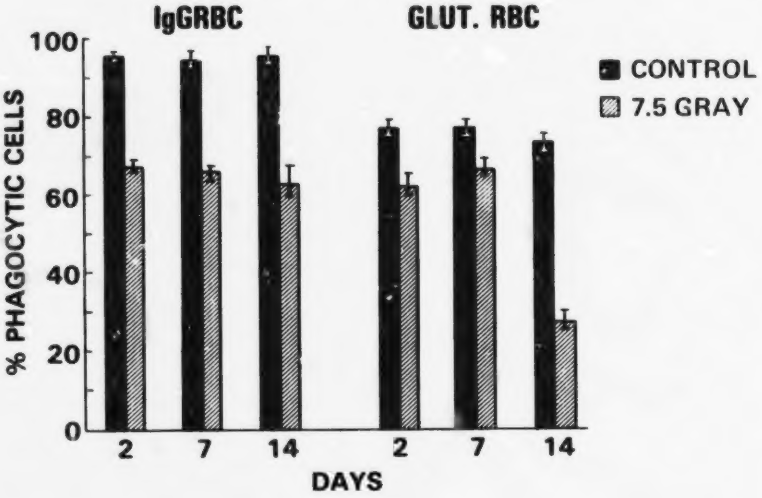

Figure 1. Percentage of cells phagocytizing or binding E(IgG) or Glut(E) at varying times postirradiation. Solid bars: Control cells. Hatched bars: Cells irradiated with 7.5 gray.

In order to determine whether this deficit is specific for Fc-mediated ingestion, the ability of cultured M0 to ingest or bind glutaraldehyde-treated E was examined. Irradiated cells exhibited a decreased binding of glutaraldehyde-treated particles (Figure 1). Cell survival, as monitored by cell number and release of lactic dehydrogenase into the medium, was less sensitive to radiation exposure than was the phagocytic ability of the culture. These data indicate that exposure to in vitro radiation produces a time-dependent decrease of both Fc-receptor-mediated and nonspecific particle ingestion by M0.

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CHANGES IN MEMBRANE POTENTIAL DURING MACROPHAGE ACTIVITIES

Principal Investigator: E. K. Gallin

Electrophysiological studies of cultured macrophages have demonstrated voltage-dependent conductances in these cells (1). These conductances have been studied directly by voltage-clamping macrophages using a single electrode voltage clamp (2). Voltage-clamp studies produced N-shaped steady-state current-voltage relationships containing a region of negative slope resistance (Figure 1).

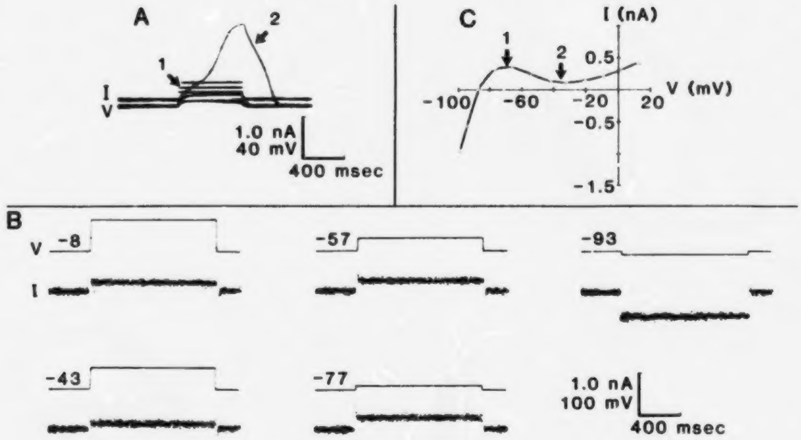

Figure 1. Voltage clamp and current clamp data from a cultured spleen macrophage. A: Current clamp records showing three voltage responses of cell to injected currents. Cell had a resting potential of -87 mV. Top tracing monitors current; bottom tracing, voltage. B: Voltage clamp data showing currents resulting from 1.2-sec step commands from zero current holding potential. C: Current-voltage plot of voltage clamp data shown in B. Steady-state currents are measured at end of step command.

Some macrophages exhibit two stable states of membrane potential that have current-voltage relationships crossing the voltage axis at three points. Outward currents that turn on at voltages of +15 millivolts or greater were noted in several cells. The addition of barium chloride to the bathing medium abolished the negative slope resistance and reduced the inward currents in response to hyperpolarizing voltage steps.

These data provide direct evidence that macrophages exhibit at least two different voltage-dependent conductances, and demonstrate that voltage clamp techniques can be useful in studying the membrane properties of leukocytes.

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2. Gallin, E. K. Voltage clamp studies in macrophages from mouse spleen cultures. Science 214: 458-460, 1981.

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INTESTINAL BLOOD FLOW AND CELLULAR DAMAGE IN CANINE AFTER ACUTE RADIATION

Principal Investigators: L. G. Cockerham, T. F. Doyle, R. B. Trumbo, and J. B. Nold

Radiation-induced early transient incapacitation (ETI) is accompanied by severe systemic hypotension, during which arterial blood pressure often decreases to less than 50% of normal. One hemodynamic compensatory mechanism is the increased peripheral resistance due to vasoconstriction. This vasoconstriction in the small intestine of the dog is disproportionately increased during hemorrhagic or endotoxic shock, and intestinal ischemia is frequent.

In an attempt to elucidate the mechanisms underlying this radiation-induced ETI and the gastrointestinal radiation syndrome, we measured the canine intestinal blood flow by the technique of hydrogen polarography, before and after exposure to gamma radiation. Systemic blood pressures, blood gases, and hematocrits were determined simultaneously.

The data obtained from 12 sham-radiated dogs and 12 radiated dogs indicated that 10 k rads of whole-body gamma radiation produced a 31% decrease in the systemic mean blood pressure, beginning within 10 minutes after radiation and lasting for at least 90 minutes. The intestinal blood flow did not decrease as anticipated, but it did exhibit an actual postradiation increase. This increase in postradiation intestinal blood flow began within 5 minutes after radiation, and lasted for at least 90 minutes. Postradiation hematocrits were 10.5% higher than those obtained before radiation and those obtained from sham-radiated subjects. Histopathological examination of the ileal mucosa revealed significant pathologic lesions in radiated animals within 2 hours after exposure.

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EFFECT OF IONIZING RADIATION ON TRANSPORT OF INTESTINAL ELECTROLYTES: MECHANISMS OF ELECTROLYTE AND FLUID LOSS

Principal Investigator: P. Gunter-Smith

A laboratory has been established and techniques have been introduced to study the mechanisms associated with the radiation-induced loss of electrolytes. Initial studies have used the response of the small intestine of an amphibian, the *Necturus*, to other secretagogues, such as theophylline, to assess the role of intracellular calcium as a common intracellular mediator of the intestinal secretion of electrolytes.

Preliminary experiments with classical calcium transport antagonists have shown little ability of these agents to inhibit the secretory response. This suggests that the intracellular events mediating the secretion may not involve only calcium, and that the events may be more complex than previously thought.

Future experiments will define the characteristics of electrolyte transport in the irradiated small intestine, and will compare those results with the present ones, in an attempt to determine the intracellular mediator of the response.

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CARDIOVASCULAR DYSFUNCTION

Principal Investigator: R. N. Hawkins

A laboratory has been established and instrumentation has been accumulated to monitor chronically instrumented rats for acute studies and long-term studies of the effects of ionizing radiation on cardiovascular dysfunction.

Techniques have been developed for the monitoring of arterial pressure, central venous pressure, mean circulatory filling pressure, and cardiac output in conscious rats. Studies have been initiated recently to establish control values in sham-irradiated rats for gain in the circulatory system, in response to hemorrhage. In addition, investigation has been started on the release of various humoral agents in chronically instrumented rats, in response to ionizing radiation.

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SCIENTIFIC SUPPORT DEPARTMENT

The Nuclear Sciences Division of the Scientific Support Department conducts research on the effects of ionizing radiation on the structure and function of different organic systems by the use of radioactive tracers and radiopharmaceuticals in scintigraphic and *in vitro* experimental models. The experiments are conducted by the use of various species of experimental animals, including rodents, canines, and subhuman primates. During fiscal year 1982, the Nuclear Sciences Division had ten active work units related to the seven areas of experimental research. The major areas of the studies performed in the Division include the following:

> Evaluation of morphological changes and function of the respiratory system with the use of radioactive gases, aerosol inhalational agents, and pulmonary perfusion radiolabeled particles, after the exposure of experimental animals to various doses of photon and neutron irradiation.
>
> Studies of the effects of ionizing radiation on the gastrointestinal system in experimental animals, with particular interest focused on mission-oriented research on radiation-induced vomiting and its prevention by the use of dopamine antagonist agents.
>
> Studies of biological indicators in the assessment of radiation damage, with particular interest to developing a biological dosimeter using red blood cell changes in technetium-99m pertechnetate-binding capacity after irradiation.
>
> Studies of the morphological sites and metabolic behavior of muscarinic receptors before and after irradiation by the use of radioiodinated quinuclidinyl-p-iodobenzilate.
>
> Experimental research on the quality control for technetium-99m generators for the purpose of determining performance and limitations of the generator.
>
> Studies of cardiovascular system function by the use of an adjustable nuclear stethoscope.

Research on the development of a computer program for positron emission computerized tomographic systems, by the use of generalized Monte Carlo analysis methods.

Morphological and Functional Changes of the Pulmonary System After Hemithoracic Irradiation

Morphology and function of the respiratory system have been studied in dogs after irradiation of the right hemithorax with cobalt-60 photons or fast fission neutrons. The experimental animals were examined by scintigraphic techniques, including lung ventilation studies (using Tc-99m-Sn-phytate aerosol and xenon-133) and perfusion studies using technetium-99m macroaggregated albumin. Ventilation and perfusion studies were performed using multiple-view techniques. The relative distribution of xenon-133, Tc-99m-Sn-phytate aerosol, and technetium-99m macroaggregated albumin were quantitated in different parts of the lungs. These experiments are being conducted in reference to a direct military relevance addressing combat performance in postirradiation syndrome in view of the observed changes of atrophy, fibrosis, and blood vessel occlusion in the irradiated lungs.

Effect of Irradiation on Gastrointestinal Function

The effects of cobalt-60 photon irradiation on gastric emptying and secretion have been studied with a military mission-oriented goal of the prevention of radiation-induced vomiting by the use of a dopamine antagonist domperidone. The studies were performed on dogs and subhuman primates. Assessment of radiation-induced vomiting and its relationship to the gastric emptying have been evaluated in dogs. Gastric fractional emptying rate acid output, gastric basal electric rhythm, and plasma endorphin levels have been studied on monkeys. The principal goals are to study radiation-induced vomiting and delayed gastric emptying, which adversely affect combat performance, and to study methods of reducing these adverse effects by administering optimal antiemetic agents.

Biological Dosimetry

One of the objectives in AFRRI's 5-Year Plan is the development of a biological dosimeter. Efforts were made to study the potential of using red blood cells as an indicator of radiation damage. Changes in their binding capacity to technetium-99m pertechnetate were investigated.

Metabolism and Morphological Localization of the Muscarinic Acid Receptors Before and After Irradiation

Another objective in AFRRI's 5-Year Plan is to study the behavior of muscarinic receptors pre- and postirradiation. Fruitful collaborative efforts with George Washington University have resulted in better understanding of receptor binding and saturation and the development of a gamma-emitting iodine-123 quinuclidinyl-p-iodobenzilate. At the same time, in-depth studies were performed to analyze the differences in imaging quality of iodine-123 produced by two different nuclear rections (p, 2n) and (p, 5n).

* * *

RADIONUCLIDE EVALUATION OF PULMONARY FUNCTION IN THE CANINE LUNG 5 YEARS POST-IRRADIATION

Principal Investigators: J. J. Conklin, F. Vieras, J. P. Jacobus, M. P. Grissom, and P. O. Alderson, *AFRRI*
E. W. Bradley, *George Washington University Hospital Center, Washington, DC*
C. C. Rogers, *Columbia-Presbyterian Medical Center, New York, NY*

Perfusion and ventilation lung studies of neutron and gamma irradiation were compared in 31 beagle dogs after hemithorax irradiation with 15-MeV neutrons or cobalt-60 gamma rays in cumulative doses of 3000, 4500, or 6750 centigray and neutron doses of fractions per week for 6 weeks. None of the dogs from the two highest neutron doses survived past 1 year. The dogs underwent serial technetium-99m MAA (macroaggregated human serum albumin) perfusion, technetium-99m phytate aerosol, and xenon-133 ventilation studies before irradiation and at 3, 6, 9, and 12 months after irradiation, with subsequent studies every 6 months through 60 months. Digital data were obtained from the posterior view of each study using a computer interfaced to a gamma camera, to determine quantitative regional function by percent distribution of lung volume, aerosol deposition, perfusion, single-breath counts, ventilation-perfusion ratios, and clearance rate of xenon-133 calculated from the time-activity curve using the fractional exchange method of Secker-Walker.

Marked early reductions were seen in perfusion and aerosol deposition in the neutron-irradiated dogs and in the group of highest gamma dose. The changes in other ventilatory parameters were modest. The aerosol studies have progressively normalized, whereas pulmonary perfusion abnormalities persist after neutron or high-gamma irradiation.

COMPARISON OF MULTIPLE VENTILATION TECHNIQUES (KRYPTON-81m, XENON-133, TECHNETIUM-99m AEROSOL) IN EVALUATION OF CANINE LUNG POSTIRRADIATION

Principal Investigators: J. J. Conklin, R. R. Eng, M. P. Grissom, and A. Durakovic, *AFRRI*
E. W. Bradley, *George Washington University Medical Center, Washington, DC*
M. E. Corral, *Uniformed Services University of the Health Sciences, Bethesda, MD*

The effects of neutron and gamma irradiation on pulmonary ventilation were compared in 17 beagle dogs irradiated to the right hemithorax with cobalt-60 or 15-MeV neutrons. Cumulative doses of 3000, 4500, and/or 6750 rads of cobalt-60 and doses of 1000 and 1500 rads of neutrons were given in four equal weekly fractions for 6 weeks. The dogs underwent technetium-99m phytate aerosol and krypton-81m and xenon-133 ventilation studies 60 months postirradiation. Comparison was made between krypton-81m, technetium-99m phytate aerosol, and xenon-133 ventilation, in single-breath, 50-second-ventilation, equilibrium, and clearance studies. Xenon clearance was calculated from the time-activity curve using the fractional exchange method of Secker-Walker. All other ventilatory parameters are expressed as ratio of counts in the right lung to number of total counts in both lungs.

All ventilation studies agreed closely, considering that they measured different aspects of pulmonary physiology. The pattern of aerosol deposition compared very closely with the krypton-81m and xenon-133 ventilation studies.

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PREVENTION OF RADIATION-INDUCED VOMITING AND ALTERED GASTRIC EMPTYING

Principal Investigators: A. Dubois, *Uniformed Services University of the Health Sciences, Bethesda, Maryland*
J. P. Jacobus, M. P. Grissom, R. R. Eng, and
M. E. Corral, *AFRRI*

The relation between radiation-induced vomiting and gastric emptying is unclear, and the treatment of this condition is not established. Therefore, we explored (a) the effect of cobalt-60 irradiation on gastric emptying of solids and liquids and (b) the possibility of preventing radiation-induced vomiting with the dopamine antagonist domperidone. Twenty dogs were studied on 2 separate days, blindly and in random order, following intravenous injection of either a placebo or 0.06 mg/kg domperidone. On a third day, they received 800 rads whole-body irradiation after either placebo ($n = 10$) or domperidone ($n = 10$). Before each study, each dog was fed chicken liver tagged in vivo with 1 mCi technetium-99m sulfur colloid (solid marker), and 1 mCi indium-111 diethylenetriamine pentacetic acid in water (liquid marker). Dogs were placed in a Pavlov sling for the subsequent 3 hours, and radionuclide imaging was performed at 10-minute intervals. Irradiation produced vomiting in 9 of 10 dogs given placebo but only in 1 of 10 dogs pretreated with domperidone ($p < 0.01$). Gastric emptying of both liquids and solids was significantly suppressed by irradiation ($p < 0.01$) both after placebo and domperidone.

These results demonstrate that radiation-induced vomiting is accompanied by suppression of gastric emptying. Furthermore, prevention of vomiting with domperidone does not alter the delay of gastric emptying produced by ionizing radiations.

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EFFECTS OF NEUTRON IRRADIATION ON RED BLOOD CELL LABELING WITH TECHNETIUM-99m

Principal Investigators: R. R. Eng, J. J. Conklin, and M. P. Grissom

The effects of in vivo and in vitro neutron irradiation on red blood cell radiolabeling with technetium-99m were studied. Blood from three dogs was irradiated with neutrons (725 rads, free-in-air dose) followed by radiolabeling with technetium-99m. The three dogs were subsequently whole-body neutron-irradiated (250 rads, midline dose), and blood samples were drawn for radiolabeling at 24, 48, 72, and 96 hours postirradiation. Blood from three control dogs was also drawn and radiolabeled on each day for comparison.

Dog blood placed in a test tube and then neutron-irradiated did not show any significant differences in radiolabeling capacity compared to nonirradiated blood (Table 1). Also, the radiolabeling capacity of RBC's from neutron-irradiated dogs at 24, 48, 72, and 96 hours postirradiation was not statistically different ($p > 0.20$) from blood of control dogs.

Table 1. Radiolabeling Capacity of Red Blood Cells

	Neutron-Irradiated*	Controls	%Δ
In Vitro	1.494 ± 0.160	1.540 ± 0.120	-3%
In Vivo			
24 hr	1.976 ± 0.270	1.708 ± 0.016	+15.0
48 hr	2.133 ± 0.141	2.136 ± 0.339	-0.1
72 hr	1.784 ± 0.213	1.533 ± 0.176	+16.0
96 hr	1.833 ± 0.130	1.838 ± 0.053	-0.3

*Figures are cpm/g RBC/mCi technetium-99m used x 10^9

These results indicate that up to 96 hours after neutron irradiation, dog RBC's are very radioresistant in terms of technetium-99m-binding capacity. Also, the results indicate that RBC-binding capacity to technetium-99m is not influenced by how dog blood is neutron-irradiated, whether it be *in vitro* or *in vivo*. One can conclude from these results that gated blood pool studies using *in vivo* radiolabeled technetium-99m RBC's on fission neutron-irradiated dogs can be performed up to 96 hours postirradiation without difficulty from the standpoint of RBC-radiolabeling capacity.

IMAGING POTENTIAL OF IODINE-123-LABELED QUINUCLIDINYL p-IODOBENZILATE

Principal Investigators: J. J. Conklin, M. P. Grissom, and R. R. Eng, *AFRRI*
W. C. Eckelman and R. C. Reba, *George Washington University Medical Center, Washington, DC*

This study continues our development of a muscarinic cholinergic receptor-binding radiopharmaceutical. Tissue distribution studies of iodine-125-labeled quinuclidinyl p-iodobenzilate (p-IQNB) were studied in rats in conjunction with displacement using unlabeled QNB, and quantitated as % dose/gram. This radioiodine study was to confirm biodistributive properties of p-IQNB found in our tritiated QNB studies before *in vivo* imaging. Heart-to-blood ratios were 8.7 and 5.6 at 1/4 and 2 hr, respectively. Displacement by unlabeled QNB was seen at both times. At 2 hr, the lung-to-blood ratio was 29.2 and the heart-to-lung ratio was 0.19.

Pancreas-to-blood ratio was 13.2 and 37.7 at 1/4 and 2 hr, and pancreas-to-liver ratio was 1.5 and 1.7, respectively. In addition, the pancreas-to-liver ratio for displacement studies did not differ significantly from that in nondisplacement studies. The midbrain-to-blood ratio at 2 hr was 6.7, and the cerebellum-to-blood ratio was 1.63 with displacement using unlabeled QNB. In vivo imaging in dogs (n = 3) with iodine-123-labeled p-IQNB showed excellent visualization of several organ systems, especially the midbrain.

Using planar imaging, the discrimination between heart and lung as well as between pancreas and liver was difficult. Single photon emission computed tomography (SPECT) will be used in the near future to further enhance discrimination of heart and pancreas. These diastereoisomers of p-IQNB show significant promise as a midbrain radiolabel of muscarinic receptors. The results encourage continuing studies of heart and pancreas using SPECT techniques and/or other QNB moieties.

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ALKYL DERIVATIVES OF QUINUCLIDINYL BENZILATE: POTENTIAL IMAGING AGENTS

Principal Investigators: R. R. Eng, J. J. Conklin, and M. P. Grissom, *AFRRI*
W. C. Eckelman and R. C. Reba, *George Washington University Medical Center, Washington, DC*

Tissue distribution studies of tritiated quinuclidinyl benzilate (QNB) were studied in rats in conjunction with its displacement by nonradiolabeled alkyl derivatives of QNB. This technique was used as a screening procedure for potential muscarinic imaging agents.

In the heart, 90%+ tritiated QNB was displaced by the butyl (R = butyl) and cyclopentyl (R = cyclopentyl) derivatives of QNB, while only 25%-42% of the tritiated QNB was displaced in the lung by these derivatives. Blood levels of tritiated QNB remained relatively constant for the displacement studies. These results indicate that these alkyl derivatives may localize in cardiac muscarinic receptors as well as or possibly better than QNB, but may localize in lung tissue much less than tritiated QNB. Therefore, higher heart/lung ratios might be observed for these alkyl derivatives.

In the pancreas, 69% and 56% of the tritiated QNB were displaced by the butyl and cyclopentyl derivatives of QNB, respectively, while liver concentration of tritiated QNB remained fairly constant for butyl derivative displacement study. Along with QNB, its alkyl derivatives may be potentially useful as pancreas-imaging agents.

In the cerebellum, at least 70% of the tritiated QNB was displaced by the butyl and the cyclopentyl derivatives of QNB. In the midbrain, at least 50% of the tritiated QNB was displaced by the butyl derivative. In conclusion, these results indicate the potential usefulness of alkyl derivatives of QNB as imaging agents of the heart, brain, and pancreas.

* * *

IN VIVO DISPLACEMENT BY MUSCARINIC ACETYLCHOLINE RECEPTOR (m-AChR) BINDING LIGANDS

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We have attempted to develop an efficient strategy for the choice, synthesis, in vitro analysis, and animal distribution studies of receptor-binding radiotracers. Because of the special requirements for receptor-binding radiotracers (high effective specific activity, high chemical and radiochemical purity, low non-receptor binding), the choice is crucial. To the goal of preparing a gamma-emitting 3-quinuclidinyl benzilate (QNB) derivative, we have synthesized a number of nonradioactive derivatives substituted with stable isotopes of the routinely used radionuclides iodine-123, bromine-77, bromine-75, and fluorine-18.

These stable halogenated derivatives of QNB were used in in vivo displacement studies with tritiated hydrogen QNB to determine their ability to compete with the parent structure QNB for m-AChR and thus give an indication of their own distribution. Because making each derivative with iodine-123, bromine-77, bromine-75, or fluorine-18 would be an overwhelming task, we thought that this approach (in vivo displacement) would be an efficient strategy to determine which radiotracer to pursue.

Fifty nmol of each halogen derivative of QNB was coinjected with tritiated hydrogen QNB in rats. Meta- and parafluorobenzilate of 3-quinuclidinyl are the most potent, followed by the two bromo derivatives and finally the two iodo derivatives. These displacement values are in general agreement with the affinity constants determined by in vitro assay (Tables 1, 2 and Figure 1). Experimental values for the concentration of tritiated hydrogen QNB in the organ with (B) and without (B_o) coinjected displacer were plotted using a logit-log transformation. The equation for the heart is

$$\text{logit } Y = 4.93 - 0.999 \ln \text{RBI} \quad (r^2 = 0.77)$$

where logit $Y = \log (B/B_o) / (1 - B/B_o)$ and RBI is the relative binding affinity. More nonradioactive QNB is required to displace the same concentration of tritiated hydrogen QNB from the corpus striatum than from the heart. This indicates higher concentration of receptor in the brain and/or less transfer across the blood-brain barrier.

Table 1. Relative Binding Index (RBI) of QNB Derivatives Obtained From *In Vitro* Studies Using Rat Heart and Rat Brain and *In Vivo* Distribution of Tritiated Hydrogen QNB and Co-injected Derivative

R	RBI(Heart)	%Dose/g Heart*	RBI(Corpus Striatum)	%Dose/g Corpus Striatum*
C_6H_5(QNB)	100	.092 ± .013	100	.389 ± .064
C_5H_9	155	.206 ± .019	76	.210 ± .021
C_6H_{11}	66	.446 ± .043	60	.460 ± .079
C_4H_9	55	.378 ± .093	—	.336 ± .055
4-FC_6H_4	55	.363 ± .053	71	.454 ± .031
3-FC_6H_4	95	.284 ± .020	83	.508 ± .086
4-BrC_6H_4	11	1.88 ± .173	71	.491 ± .102
3-BrC_6H_4	8	1.23 ± .133	62	.515 ± .067
4-IC_6H_4	23	2.12 ± .067	67	.722 ± .134
3-IC_6H_4	9	2.78 ± .385	40	.627 ± .086

* Dose/g in organ 2 hr after injection of tritiated hydrogen QNB and 50 nmol of derivative. B/B_0 is calculated by dividing the %dose/g in the presence of derivative by the %dose/g in the presence of saline.

Table 2. Distribution of I-125 pIQNB at 1/4 and 2 Hours in Rat

		% Dose/g ± SD				
Displacer	Time	Blood	Lung	Heart	Cerebellum	Corpus Striatum
Saline	1/4 hr	.118 ± .009	5.57 ± .811	1.03 ± .235	.180 ± .042	.222 ± .047
50 nmol QNB	1/4 hr	.056 ± .018	4.32 ± 1.94	.45 ± .134	.139 ± .047	.178 ± .068
Saline	2 hr	.049 ± .009	1.43 ± .405	.274 ± .016	.080 ± .024	.333 ± .093
50 nmol QNB	2 hr	.038 ± .003	.961 ± .174	.124 ± .013	.030 ± .002	.069 ± .005

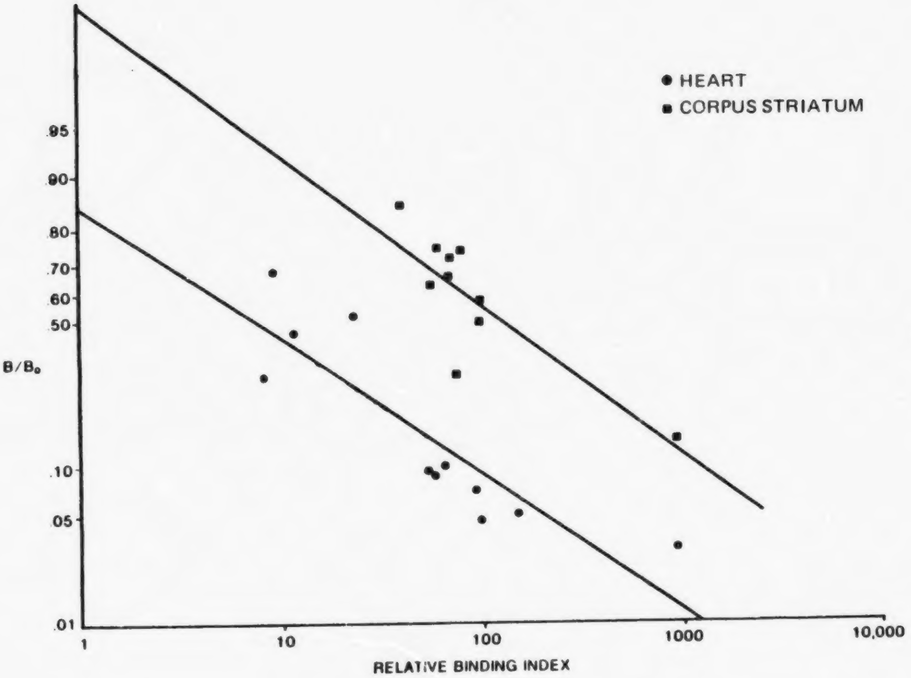

Figure 1. Competitive binding of tritiated hydrogen in the heart and corpus striatum

The distribution of quinuclidinyl p-iodobenzilate (pIQNB) labeled with iodine-125 was studied to determine the correlation between the in vivo displacement study and radiolabeled distribution studies. Iodine-125-labeled p-IQNB gives reasonable heart-to-blood ratios, but high lung concentration is also evident. The radioactivity is displaceable by 50 nmol of QNB from the heart, the cerebellum, and the corpus striatum, indicating that pIQNB is binding to m-AChR at least in part in these organs. Using iodine-123-labeled pIQNB, we were able to visualize the heart and the cerebrum of dog. The major route of excretion is by the hepatobiliary system.

In the case of these m-AChR-binding ligands, the in vivo displacement studies correlate with the in vitro affinity constants, indicating that transport to the receptor is not a major factor in determining the distribution, but the affinity constant to the receptor is. In general, the in vivo displacement study using nonradioactive halogenated derivatives is an important screening test for receptor-binding radiotracers.

* * *

TRITIATED (-) QUINUCLIDINYL BENZILATE DISPLACEMENT STUDIES IN RATS: MUSCARINIC AGENTS

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A series of muscarinic compounds were studied in the rat by means of their ability to displace tritiated (-) quinuclidinyl benzilate (QNB) in various organs. At 2 hours postinjection, the animals were sacrificed and tissues analyzed by liquid scintillation techniques.

Of all the agents tested, none had the displacement capacity of QNB in either cardiac or brain tissues studied (see Tables 1, 2). In lung, nitrogen-methyl scolpolamine (nitrogen-mSCO) had equivalent displacement activity to QNB ($p > 0.8$) (Table 1). In the pancreas, nitrogen-mSCO ($p > 0.2$) and scopolamine (SCO, $p > 0.9$) were of equivalent displacement strength to QNB (Table 3). A study of nonlabeled QNB demonstrated a progressive displacement of tritiated QNB from each organ except liver at four different displacement concentration levels.

Table 1. Tritiated (-) QNB in Rat Lung and Heart (% Dose/g ± S.D.)

Displacer	Blood (B)	Lung	Heart (H)	H/B
QNB	0.217 ± 0.052	0.295 ± 0.072	0.244 ± 0.033	1.1
N-mSCO	0.074 ± 0.006	0.286 ± 0.047	0.453 ± 0.051	6.1
SCO	0.104 ± 0.009	0.339 ± 0.061	1.790 ± 0.247	17.2
BEN	0.062 ± 0.010	0.390 ± 0.051	3.920 ± 0.372	63.2
Br ATR	0.061 ± 0.012	0.436 ± 0.092	3.250 ± 0.310	53.3
COG	0.058 ± 0.008	0.492 ± 0.089	4.310 ± 0.347	74.1
CARB	0.069 ± 0.007	0.448 ± 0.064	4.630 ± 0.785	67.2

Table 2. Tritiated (-) QNB in Rat Brain (% Dose/g ± S.D.)

Displacer	Blood (B)	Mid-Brain (MB)	Cerebellum	MB/B
QNB	0.217 ± 0.052	0.464 ± 0.052	0.142 ± 0.016	2.1
N-mSCO	0.094 ± 0.006	0.641 ± 0.090	0.507 ± 0.068	8.6
SCO	0.104 ± 0.009	0.546 ± 0.050	0.304 ± 0.034	5.2
BEN	0.062 ± 0.010	0.542 ± 0.046	0.403 ± 0.033	8.7
Br ATR	0.061 ± 0.012	0.517 ± 0.084	0.434 ± 0.066	8.4
COG	0.058 ± 0.008	0.656 ± 0.060	0.596 ± 0.132	11.2
CARB	0.069 ± 0.007	0.710 ± 0.148	0.608 ± 0.108	10.3

Table 3. Tritiated (-) QNB in Rat Liver and Pancreas (% Dose/g ± S.D.)

Displacer	Blood (B)	Liver	Pancreas (P)	P/B
QNB	0.217 ± 0.052	0.337 ± 0.052	0.228 ± 0.039	1.1
N-mSCO	0.074 ± 0.006	0.236 ± 0.028	0.206 ± 0.029	2.8
SCO	0.104 ± 0.009	0.253 ± 0.024	0.230 ± 0.034	2.2
BEN	0.062 ± 0.010	0.201 ± 0.034	0.938 ± 0.167	15.1
Br ATR	0.061 ± 0.012	0.191 ± 0.025	0.646 ± 0.142	10.1
COG	0.058 ± 0.008	0.177 ± 0.051	0.774 ± 0.106	13.1
CARB	0.069 ± 0.007	0.207 ± 0.021	0.875 ± 0.160	12.7

* * *

IMAGING CHARACTERISTICS OF (p,2n) AND (p,5n) PRODUCED IODINE-123

Principal Investigators: M. P. Grissom, R. R. Eng, J. J. Conklin,
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Imaging studies using three gamma camera systems were performed using five phantom arrangements wherein each phantom was loaded with either iodine-123 from (p,2n) or (p,5n) production sources. Cameras used included a MEDX-37PMT SFOV (standard field of view), an Elscint LFOV (large field of view), and a GET 400 T SPECT (single photon emission computerized tomography) system. For the SFOV and LFOV studies, three-line sources, with each line separated by 2.5 or 5.0 cm, and the BRH multiple-line source were used. For the SPECT studies, a modified BRH multiple-line source phantom and an interfilled quad cylinder phantom were used. A variety of low-energy (140 KeV) and higher energy collimators were examined. In addition, multichannel analyzer analyses were performed in order to identify principal contaminants. As noted by the vendor, iodine-124 was the major (p,2n) iodine-123 production contaminant. No significant contaminant activity was found in the (p,5n) samples.

Several artifacts such as Moire patterns were observed using medium- and high-energy collimators in the imaging of (p,2n) iodine-123 phantoms. Also, high-sensitivity penalties existed with imaging times increased two to four times compared to low-energy high-sensitivity collimators. Due to the relatively high cost of iodine-123, we found (p,5n) iodine-123 to be a superior imaging isotope for SPECT studies. Due to our low yields of iodine-123-labeled agents as well as the count poor nature of emission computerized tomography sections, we have found (p,5n) iodine-123 to be the radionuclide of choice for our applications in thoracic and cephalic imaging.

* * *

QUALITY CONTROL PARAMETERS FOR TECHNETIUM GENERATORS

Principal Investigators: R. R. Eng, C. J. Munno, S. I. Miller, M. P. Grissom, and J. J. Conklin

Technetium-99m generators are an important source of radioisotopes for nuclear medicine facilities. Quality-control procedures are performed to insure maximum patient safety, such as detection of molybdenum-99 breakthrough and/or alumina breakthrough. In addition, limulus amebocyte lysate tests detect pyrogen, and thin-layer chromatography monitors unbound technetium-99m in a radiopharmaceutical preparation.

Two quality control factors little used in a radiopharmacy, requiring calculations and radiopharmaceutical data not normally maintained in a radiopharmacy, are generator elution efficiency and number of technetium atoms (Tc-99m + Tc-99) in a radiopharmaceutical preparation. Elution efficiency not only monitors daily generator performance but also aids in selecting commercially available generators for maximum return on a cost-per-unit-activity basis. Radiopharmaceuticals have an activity limit of technetium-99m activity that can be bound for reasons of radiolytic decomposition, amount of reducing agent available, and number of sites for technetium-99m binding.

We have formulated a computer program to calculate generator elution efficiencies and the number of technetium-99m plus technetium-99 atoms in a particular elution. Other generated data are activity of technetium-99m available for elution, current molybdenum-99 activity in the generator, and residual number of technetium-99m plus technetium-99 atoms from each elution period remaining on the generator. Input data are date and time of elution, activity of technetium-99m elution, and calibration time and time of the generator. Also, manufacturer's loading, elution, and quality-control procedures preshipment should be known, to account for significant activity levels of technetium-99m at the specified calibration time.

* * *

CHARACTERISTICS OF OUTPUT COUNTING LEVELS USING AN ADJUSTABLE NUCLEAR STETHOSCOPE

Principal Investigators: R. R. Eng, J. J. Conklin, and M. E. Corral

Adjustable lower and upper controls were added to a single-probe nuclear stethoscope (Bios) in order to examine their characteristics for use in dual-isotope heart studies. A 10K ohm ten turn pot was used for each control. The upper control served to adjust the high voltage to the photomultipler tube. In the conventional nuclear stethoscope, the high voltage is set at 1100 V for the adjustable nuclear stethoscope. The voltage could be adjusted from 500 to 1500 V. The lower control served to vary the amplitude for the pulse height cutoff, thus functioning as a gain potentiometer. As the control setting was advanced, higher energy events were cut off. Previous nuclear stethoscope additions using a single switch to control the lower cutoff allowed only indium-111 or indium-113m to be seen (90%) while blanking out technetium-99m. For the variable controls function test, 80 µCi of each technetium-99m and indium-111 in syringes were studied sequentially at varying pot settings in the patient monitor mode.

It was seen that at setting 990 with high voltage at full scale, 97.5% of the technetium-99m counts had been removed, while 52.6% of indium-111 counts remained. It appeared that the lower energy indium-111 photon was being lost with technetium-99m counts due to the limited energy response of this system (which requires nearly full-scale setting of the lower pot in order to reduce technetium-99m counts). The dual pot adjustment will likely be useful only in dual-isotope studies with one isotope of energy 200 keV and the other of energy 150 keV.

* * *

A GENERALIZED MONTE CARLO ANALYSIS OF THE DESIGN AND PERFORMANCE PARAMETERS OF POSITRON-IMAGING SYSTEMS

Principal Investigators: F. Atkins, J. J. Conklin, and A. Durakovic

The primary objective of the research project is to develop a comprehensive computer program and to compare various positron emission computerized tomographic (PECT) systems. This research is an extension of the current research projects in the Nuclear Sciences Division using single-photon emission computerized tomography. The essential value of PECT is that this methodology permits in vivo, regional, and noninvasive investigations of many biochemical processes essential to life.

There are several basic PECT designs, each with its own strengths and weaknesses. It would be useful to have a uniform code to predict performance characteristics of each design configuration. Monte Carlo methods would be used in developing such simulation codes. In addition to the hardware evaluation capability, the simulations would also provide projection data for evaluation of the reconstruction algorithms (software). The ability to evaluate a system's electromechanical and software capability is a tremendous advantage antecedent to the purchase of expensive state-of-the-art equipment.

Computer subroutines for performing a number of operations needed as part of the Monte Carlo simulations have been written and encoded on the PDP 11/70 system. These include pseudorandom number generations and also routines for sampling from exponential, isotropic, coherent, and incoherent scattering distributions. A program to simulate the response of a positron camera wing to large area detectors for a point source at the center of a spherical medium has also been written and encoded. The next step will involve testing the individual subroutines, and evaluating the collective performance for the particular design indicated above.

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